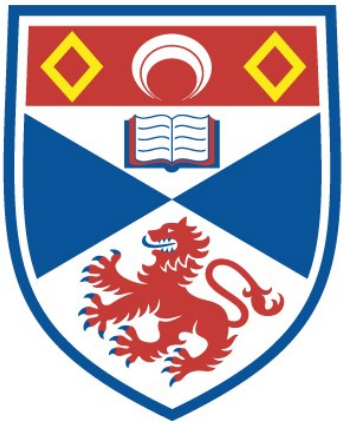
A NEUROPHYSIOLOGICAL AND BEHAVIOURAL STUDY ON CERTAIN ASPECTS OF SENSORY PERCEPTION IN 'OPHIURA OPHIURA' (L.) (ECHINODERMATA, OPHIUROIDEA)

Andrew Moore

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1986

A NEUROPHYSIOLOGICAL AND BEHAVIOURAL STUDY ON CERTAIN ASPECTS OF SENSORY PERCEPTION IN Ophiura ophiura (L.) (ECHINODERMATA, OPHIUROIDEA).

BY

ANDREW MOORE

A thesis presented for the degree of Doctor of Philosophy at the University of St. Andrews.

Gatty Marine Laboratory,
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ABSTRACT

Electrophysiological recordings were made from the radial nerve cord of the brittlestar Ophiura ophiura in response to stimulation by certain environmental parameters. Extracellular suction electrodes attached to the ectoneural portion of the radial nerve cord recorded unitary potentials in response to photic, mechanical and chemical stimulation. O. ophiura is highly sensitive to reductions in the ambient light level or shadow, the greatest response being to the rapid onset of a dense shadow on the tip of the arm. Parallel behaviour studies indicate that this same stimulus resulted in the cessation of any type of activity in the unrestrained animal. The "freezing" of the brittlestars movements at the onset of such a shadow stimulus, is suggested to be a means of escaping detection by a predator. The range that the brittlestar is able to detect in the mechanical mode extends from the nearfield of a vibrating source, through the far field propagated pressure wave, to movement of the aquatic medium as a whole in the form of water currents. The behavioural responses of unrestrained animals to stimulation in the same frequency range is described. The presence of an interoreceptor located within the spines, which is responsible for some mechanical sensitivity is inferred from electrophysiological recordings in response to the movement of individual arm spines. Using neurophysiological techniques, O. ophiura could be shown to detect consistently a range of amino acids at concentrations as low as 2×10^{-12}M. as in the case of L-Leucine. Parallel behaviour experiments were inconsistent and indicate the inadequacies of using behavioural criteria to demonstrate the sensory discriminatory abilities of echinoderms. An anatomical study of the podia, spines and general surface of O. ophiura using the scanning and transmission electron microscopes, describe various types of ciliated cells which may function as receptors. The relationship of these structures to the sensory abilities of the brittlestars detected physiologically is described.

I dedicate this thesis to my wife Katherine.

DECLARATION.

I, Andrew Moore, hereby certify that this thesis has been composed by myself, that it is a record of my own work, and that it has not been accepted in partial or complete fulfilment of any other degree or professional qualification.

SUPERVISOR'S CERTIFICATE.

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the Degree of Ph.D.

I was admitted to the Faculty of Science of the University of St. Andrews under the Ordinance General No.12 on 1st. October 1982, and as a candidate for the degree of Ph.D. on 1st. October 1982.

ACKNOWLEDGEMENTS.

Thanks to Dr. J.L.S. Cobb under whose supervision this work was carried out, for his infinite patience guidance and enthusiasm throughout the last 3 years.

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Ophiura ophiura

CONTENTS

INTRODUCTION

INTRODUCTION

The echinoderms are a wholly marine group of animals basically pentaradiate in construction and derived from an original bilaterality. Lack of any cephalization has resulted in an essentially non-centralised nervous system and few organized receptor organs. Receptor structures are present but generally widespread over much of the surface, podia and spines in echinoderms.

The nervous system is circumorally disposed with extensions into the arms and ambulacra. It is structurally a relatively simple system with much of the nervous system retaining a position close to the epidermis. Some concentration of the nervous elements has occurred resulting in the formation of the radial nerve cords and circumoral nerve ring. The radial nerve cords are swollen in each arm segment to form a ganglion and a number of nerve branches extend to innervate the podia, spines and integument. The ganglia contain extensive areas of neuropile whilst the interganglionic regions consist of tracts of fibres aligned in parallel.

Despite the relative ease with which the nervous system can be reached, neurophysiological studies on echinoderms are scarce. (see Takahashi, 1964, Sandeman 1965, Millott and Okumura 1968, Binyon and Hasler 1970, Podol'skii 1972, Tuft and Gilley 1985). This has been

due to the small size of echinoderm neurones which has made it almost impossible to record electrical activity from single units within the nerve cords.

In contrast there is a very large literature on the behaviour of the phylum Echinodermata and this was reviewed by Reese (1966). A more recent review on the subsequent work has not yet been carried out. A great deal of attention has been devoted to using changes in behaviour to monitor the sensory abilities of these animals to perceive their environment. One aspect of this research, namely chemoreception, has recently been reviewed by Sloan and Campbell (1982) who drew attention to the sometimes contradictory and almost anecdotal nature of the evidence.

However, Brehm (1977) working on the Californian brittlestar Ophiopsila californica showed it was possible to record unitary potentials from the radial nerve cord in response to electrical stimuli. He also reported the presence of ectoneural axons within these brittlestars that were larger than those present in the other four classes of echinoderms and suggested that it was from these the activity was recorded. This was the first indication of the possibilities of using ophiuroid preparations for the study of the neurophysiology of echinoderms.

Subsequently Stubbs and Cobb (1981, 1982) and Cobb and Stubbs (1981) carried out a detailed anatomical investigation on the nervous system of a British species of brittlestar, O. ophiura and showed that it possesses a system of giant fibres within both the ectoneural and hyponeural tissue of the nerve cord. In addition Stubbs (1982a) carried out a preliminary study, using suction electrodes, on the photic response in this species and was able to record bursts of single unit activity from the ectoneural nervous system of both the radial nerve cord and the circumoral nerve ring in response to increase and decrease in the ambient light level. This indicated that neurophysiological studies on the sensory discriminatory abilities of ophiuroids were possible using extracellular suction electrodes.

The work in this thesis is concerned with the abilities of Ophiura ophiura to detect various sensory parameters commonly encountered in its marine habitat. Simple extracellular suction electrodes attached to the ectoneural portion of the radial nerve cord recorded the responses of the animal to photic, chemical and mechanical stimulation. The thesis is divided into four chapters. The first deals with a detailed study of the photic response. The results indicate that the brittlestars are highly sensitive to any decrease in the light level and a complex burst of single unit activity is recordable from the radial nerve cord to the onset of a shadow. The animals response to shadows of differing

length, density and rate of production are described. Increases in the ambient light level, the light `ON' response, are also detailed. O.ophiura is shown to be most sensitive to deep shadows produced quickly on the distal portions of the arms. Parallel studies on the behaviour of the ophiuroid in response to shadow are described and show that all movement of the animals immediately ceases at the onset of the stimulus. This `freezing' is discussed together with the neurophysiological results and is suggested to have evolved as a anti-predator response, a means of escaping detection from large predators such as flatfish.

The second chapter deals with chemoreception. Recently several workers have examined the constituents of prey items that are most potent in eliciting feeding behaviour in certain echinoderms. (Zafiriou 1972, Valentincic 1973, 1979, Reimer and Reimer 1975). Amino acids in particular have been demonstrated to release appetitive behaviour. In this chapter O.ophiura is shown to be highly sensitive to ten amino acids and Betaine. The minimum threshold of response is shown to be consistent between neurophysiological preparations and detection of as little as 2×10^{-12} M. concentration is exhibited for the amino acid L-Leucine. Behavioural experiments show that the response of the brittlestars to the same minimum thresholds of amino acid concentrations, are however inconsistent and emphasise the limitations in using this approach to define the sensory descriminatory abilities of echinoderms. The behaviour of the

brittlestars to the introduction of food items into the experimental tanks is described and the results show that different responses occur as the amount of food present increases. A hierarchy of behaviour dependant upon the level of stimulus input is therefore proposed.

The third chapter is a detailed study of the mechanosensory abilities of the brittlestar and its response to various frequencies of vibration, magnitudes and rates of movement of waves and water flow. The neurophysiological results indicate the sensitivity of O.ophiura to movement within the aquatic environment. Behavioural experiments using comparable stimuli show that different responses occur at different levels of stimulus. In general the brittlestars are attracted to low frequency vibrations, and slow movement of the surrounding water. High frequency vibrations and disturbances close to the animals results in the `freezing' of movement, similar to the shadow response. This is again suggested to be as an anti-predator response. Experiments on individual lateral spines indicate that removal of the epithelium and cell surface structures does not diminish neuronal activity recordable from the radial nerve cord in response to the movement of these spines. This is the first physiological evidence for the presence of an interoreceptor within the echinoderms. It is suggested that movement within the environment is detected by the interoreceptor after physical deflection of the spines. The possibility that this permits the animals to perceive its orientation in

relation to the physical environment is discussed.

The last chapter is an anatomical study using the scanning and transmission electron microscopes of the podia, spines and general surface of the brittlestar. It describes possible receptors located on these structures and relates the receptors to those described in other species of echinoderms and with the sensory abilities of O. ophiura detected physiologically.

THE ANIMAL: COLLECTION AND MAINTENANCE.

Ophiura ophiura is a small subtidal brittlestar found around the shores of the Mediterranean, Atlantic, English Channel, North Sea and West Baltic. It is common from the lower shore down to 200M. on hard sandy substrates, usually buried with only the arm tips extended into the water column. The disc diameter may range in size from 2mms, up to 3.5cms. The majority of the specimens used in this study had a disc diameter of between 1.2-1.5cm. These were collected by beam trawl in the St. Andrews Bay area, Scotland, from a depth of 6 metres. Larger specimens with disc diameters up to 3.0cm. were supplied by the University Marine Biological Station, Millport, Isle of Cumbrae. In the laboratory the animals were maintained in sand bottomed circulation tanks with the water temperature between 4-10^{o}C. and in total darkness. The brittlestars were fed every 2-3 days with the tissue of Mytilus edulis. This has been shown by Feder (1981), to be readily consumed by O. ophiura under laboratory conditions. Maintained in this manner the animals survived indefinitely. Although no data was collected, it was observed that the disc diameters increased and some growth occured.

THE BIOLOGY, AND LABORATORY OBSERVATIONS ON THE BEHAVIOUR OF OPHIURA OPHIURA.

There is little information on the various aspects of the biology of O.ophiura. Forbes (1841) described the species under the generic name Ophiura texturata and listed areas of common occurance around the coasts of Britain. Mortensen (1927) again described the animal and provided information on its rate of growth. Animals reached sexual maturity after two years but took three or probably more years to reach full size at 3.5cms. The breeding season in O.ophiura occurs during the summer months around Plymouth (Narasimhamurti 1933), with plutei taken off the Baltic coast of Sweden in August and September by Mortensen (1931). Recently Feder (1981) described certain aspects of the feeding biology of these brittlestar collected off the coast of Denmark.

During the present 3 year investigation, trawls to collect specimens of O.ophiura were carried out throughout the year at approximately 4 week intervals dependant upon weather conditions. During the winter months November to March, up to 60 specimens of the ophiuroid were regularily collected in each 20 minute trawl. Throughout the rest of the year only 1 or 2 small animals: disc diameters < 5mms. were evident in each of the trawls. No success was achieved in locating O.ophiura in other areas of the Bay. Whether the

inability to collect the animal was due to their movement away from the areas trawled or the animals burying themselves deeper in the sand and avoiding capture is not known. Movements and migrations related to both the breeding season and changes in the feeding behaviour are known in other echinoderms such as asteroids (Hewatt 1937) and echinoids (Moore et al 1963). The inability to collect the animal coincides with its known breeding season (Mortensen 1927) and it is possible that O.ophiura may aggregate elsewhere to breed.

Fauna that were commonly collected with O.ophiura in each trawl included Pagurus bernhardus, Asterias rubens, Pleurenectes platessa, Limanda limanda Macropipus depurator and Crangon crangon. It may be presumed that these species are present in the same areas as the brittlestars. This is supported by evidence from Jones (1951) who demonstrated that in communities present on similar substrates off the Isle of Man, all these species were encountered with O.ophiura. P.platessa and L.limanda are known to predate upon O.ophiura (Moore 1982), but whether the other species predate upon this brittlestar is not known. Specimens of O.ophiura collected by trawl showed evidence of arm regeneration, indicating that the loss of parts or whole arms occured naturally. The presence of only arms and arm tips in the guts of P.plattesa (Moore 1982) suggest that partial predation of the brittlestars may occur.

Observations in the laboratory on the behaviour of O.ophiura in sand bottomed tanks showed the brittlestar to be negatively phototaxic. The brittlestars responded to unequel and sudden increases in illumination by burying itself in the sand or seeking another darkened enviroment. Burying behaviour involved sweeping sand across the disc area by lateral flexure of the arms or by moving downwards into the substrate using only its tubefeet. Burying was terminated when the disc and all parts of the arms except the distal fifth, which extended into the water column were covered. The extension of the arm tips upwards was very common and characteristic in this animal when quiescent. This behaviour in O.ophiura has also been reported by Feder (1981) and Stubbs (1982a). The brittlestars remained in this position until food was introduced into the tank at which point they emerged to feed. The detailed feeding behaviour in this brittlestar is described on page 41.

Specimens of the ophiuroid from which arms were removed for experimental purposes, showed no detrimental effects and regeneration of the arms was evident in all cases after a period of two weeks. However no data was collected in the present study on the rates of regeneration. Previous work by Zeleny (1903,1905) has shown that the rate of regeneration in this animal is faster the greater the number of arms removed.

CHAPTER 1

PHOTOSENSITIVITY

INTRODUCTION

A number of echinoderms are known to respond to increases in the ambient light level by changes in either the behaviour of the whole animal, (Hendler 1984) or discrete organs such as podia, (Millott 1956) and tentacles (Hess 1915). Yoshida and Millott (1959) have demonstrated that the spines of sea urchins respond to both an increase in light, the 'ON' response and a decrease in light, the 'OFF' response much of which is reviewed by Yoshida (1966). A further review on the photosensitivity of echinoids was carried out by Millott (1975), but it provided little new information on the subject.

Stubbs (1982a) carried out a preliminary study on the photic response in Ophiura ophiura using suction electrodes and was able to record electrophysiologically from the radial nerve cord the responses of the animal to light 'ON' and light 'OFF' stimuli. Stubbs demonstrated that the extracellular responses to light `OFF' stimuli could be recorded for durations of up to 25 seconds. He also showed that the pattern of these responses was conducted to all other arms in a substantially unaltered form.

Here a more detailed investigation of the photic response in Ophiura ophiura using extracellular techniques is described with particular emphasis on the light 'OFF' or the shadow response as it is referred to in this study. The neurophysiological results are correlated with the behaviour of the whole animal to increases in illumination and the shadow stimulus.

MATERIALS AND METHODS.

Electrophysiology.

Single arm preparations of the experimental animal were initially dissected to reveal the radial nerve cord (R.N.C.) by removal of five oral plates and a portion of the adjacent lateral plates. Complete arms were chosen for experimental purposes that showed no signs of regeneration. The arms were then immobilized in a perspex clamp oral side uppermost and placed in fresh seawater maintained at 5°C. Each preparation produced consistent responses for up to 36 hours. Recordings were made using conventional polythene suction electrodes with tip diameters of 100-300µm which were attached to the interganglionic region of the ectoneural portion of the R.N.C. The signal was amplified via a Neurolog A.C. preamp. and displayed on a Tektronix 565 oscilloscope. Permanent records were made by recording the signal on to a conventional stereo cassette and were played back via a Sharp stereo cassette deck (RT.10). The signals were then filmed using a Nihon-Kohden oscilloscope camera.

Each response was analysed by passing the signal through a Neurolog NL200 Spike Trigger, a discriminator for nerve spikes. Number and amplitude of recorded spikes were computed in an effort to discriminate single units associated with particular stimulus thresholds. The largest extracellular spikes recorded were about 50µv

and the inherent noise level was about 10μv. The signal from the tape-recorder was amplified by a factor of 5 before the input to the Neurolog NL200 spike trigger. The aperture was set equivalent to about a recorded 8μv and six separate window heights from approximately 10μv-50μv giving six separate apertures. The digital output was then passed through a Neurolog NL750 Averager to analyse distribution and frequency of each of the six bands of spike amplitudes. Certain factors need to be accounted for when interpreting extracellularly recorded neuronal activity in terms of amplitude and number of units present in the response. Summation or cancellation of units may occur in some cases. Binyon and Hasler (1970) in their study on the starfish radial nerve cord considered that all the compound potentials recorded were the result of the summation of smaller units. Due to the recording conditions single action potentials derived from a single neuron may appear bi- or triphasic and if so will produce an additive effect and distort the number of intermediate spikes especially at high frequencies of firing. The spike amplitude discrimination apparatus used to analyse responses in this study was able to discriminate units firing at high frequencies (KHz). Analysis of high speed film of recordings were made and showed that the majority of spikes were discrete. (see Fig. 1). Analysis of such spike trains showed that only within the initial high frequency bursts of the longer duration responses was there some evidence for summation. Intracellular recordings made simultaneously with those recorded extracellularly (Cobb pers. comm.)

show a consistent one to one correlation of spikes. Summation was thus not considered an important factor in the analysis of spike trains.

Experimental Procedure.

The photic stimulus was presented to the oral side of the distal five arm segments of the clamped preparation, in the form of a light spot 1.5mm in diameter. The light source was a 1.2V lens tipped bulb run from a single cell of a Nife Battery via a 2mm. diameter fibre optic cable (Barr and Stroud Ltd). Stubbs (1982a) quantified the light levels detectable by this species. In these experiments no attempts were made to quantify the light levels. As long as 1 hour was allowed for acclimation at the the light level used, consistent results were obtained. The shadow stimulus was produced using a shutter system between the light source and the fibre optic cable, in conjunction with a Tektronix Type 161 Pulse Generator. A shadow of duration of between 10m.sec. and 10 seconds could be produced. A period of 10 minutes was allowed between each stimulus to prevent habituation of the response. All experiments were carried out in total darkness.

RESULTS.

A shadow produced on the arm tip resulted in a burst of single unit activity that was recordable from the R.N.C. of the brittlestar (Fig.1a). The response was characterized by an initial complex burst of spikes preceding a train of single unit potentials with evidence of a pattern of spike bursts. The largest spikes recorded were about 50μv with the inherent noise level about 10μv. Binyon and Hasler (1970) in their work on the electrophysiology of the starfish R.N.C. maintained that the majority of spikes were the result of summation of a number of smaller units. Responses filmed at high speed, showed that the majority of the units are discrete and not caused by summation (Fig.1b). The responses, from over 25 preparations, were consistent in the number, frequency, amplitude and duration of potential for any given shadow stimulus (Fig.2). Habituation to a 0.5 second light 'OFF' stimulus repeated at intervals of 1 second occurred after 70 trials. The size and number of spikes decreased as the stimulus number increased from a complex burst of potentials of around 45μv to two and sometimes single spikes of amplitude 20μv (Fig.3).

FIG. 1

a) Typical extracellular response recorded from the radial nerve cord of O. ophiura to a shadow stimulus (arrow). b) Part of the upper trace filmed at higher speed showing that the majority of the single units are discrete and not caused by summation of units.

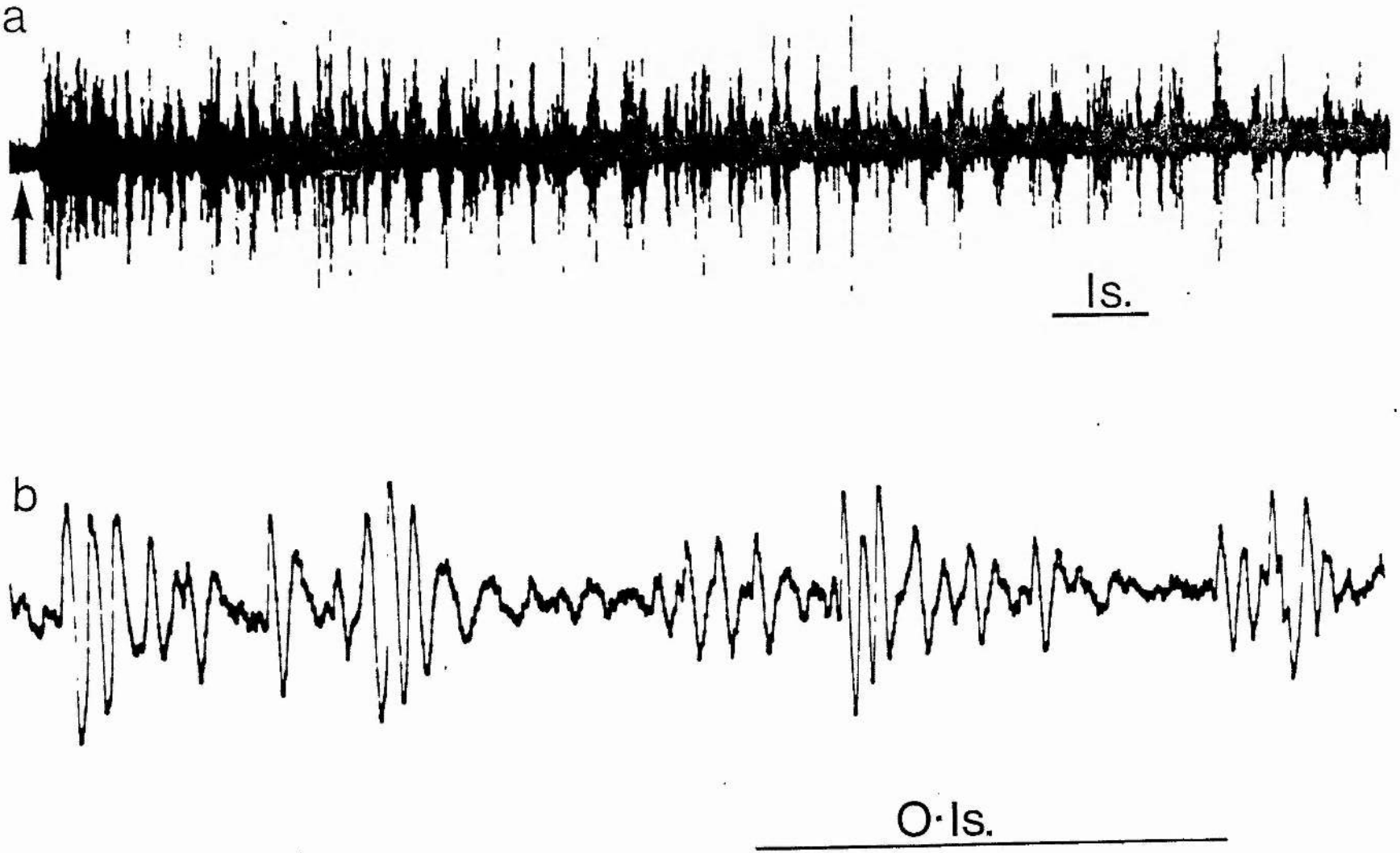

a
1s.
b
0·1s.

FIG. 2.

Extracellular recordings to show consistency of response to different shadow duration stimuli. (arrow). Shadow durations of a) 5 seconds. b) 0.7 second. c) 0.1 second.

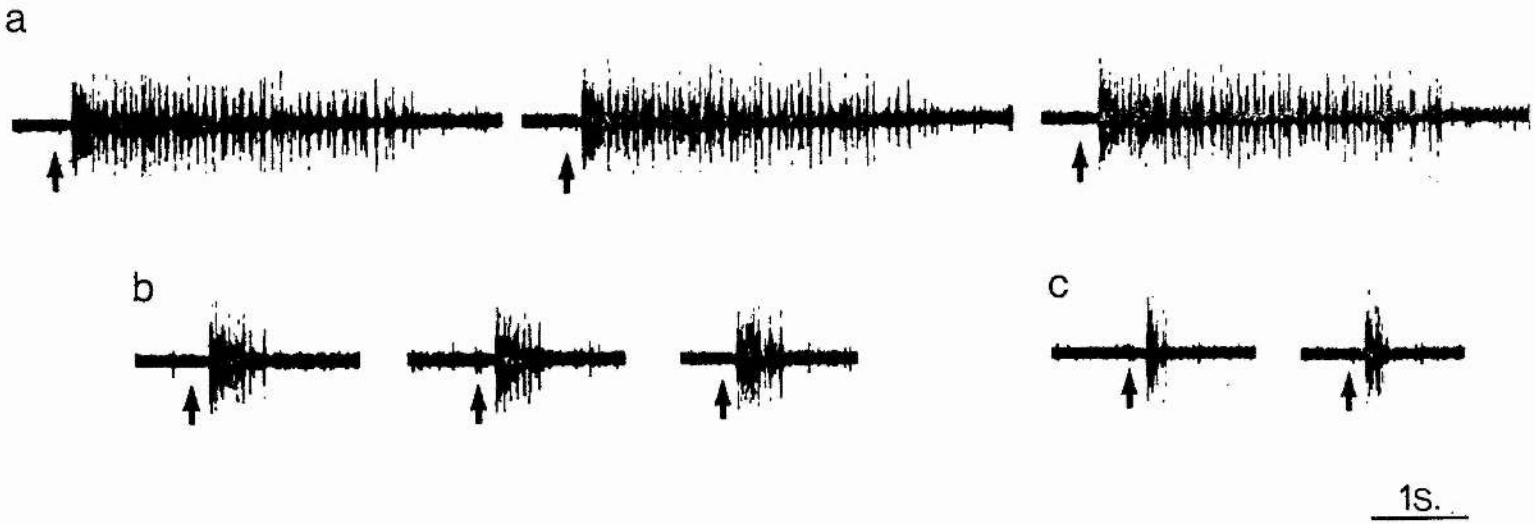
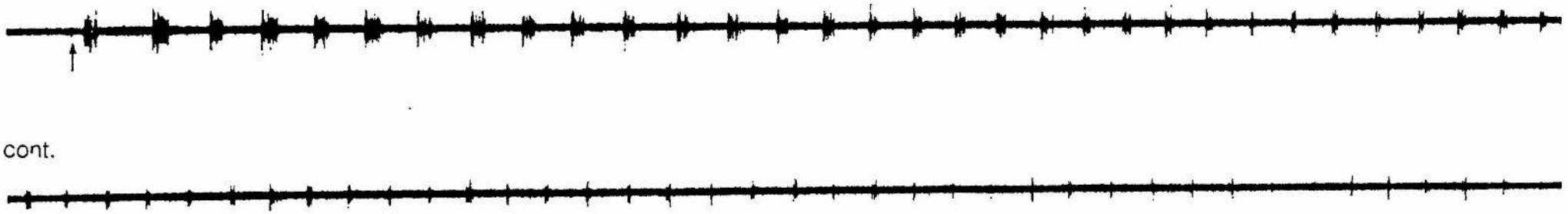

a
b
c
1s.

FIG. 3.

Repeated shadow stimuli. Responses recorded to a 0.5 second shadow stimulus repeated at intervals of 1 second. The size and number of spikes in the responses decrease with repeated stimulus. Largest spikes are approx. 40μv. Arrow marks the start of stimulation.



cont.

1s.

Shadow Duration.

Experiments were carried out to examine the responses of the animal to different shadow durations. Shadow durations ranging from 0.03 seconds to 5 seconds produced, in 25 seperate experiments, responses that were consistent in number, frequency, size and duration of spiking unit for each particular duration (Fig.4). Spikes of small amplitude were by far the most numerous in all responses (Fig.5). The pattern of response obtained to a shadow duration of 0.7 seconds was similar to the initial 0.7 seconds of a 5 second shadow in much the same way as the pattern of response from a 0.1s shadow stimulus resembled the initial pattern of both the 5s and 0.7 second shadow responses (Fig.2). No activity was elicited from the R.N.C. to shadow durations of below 0.03 seconds. No response was recorded once the arm tip was reilluminated.

Shadow Density.

The brittlestars ability to discriminate between shadows of differing densities was examined. The shadow density has been defined in this study as the relative ambient light level produced on the surface of the arm tip. Increasing the density of the shadow produced on the arm tip, elicited an increase in the response recorded from the R.N.C. of the animals. Shadows of

FIG. 4.

Responses recorded to a range of shadow durations from 3 milliseconds to 5 seconds duration. Arrows indicate initiation of shadow stimulus.

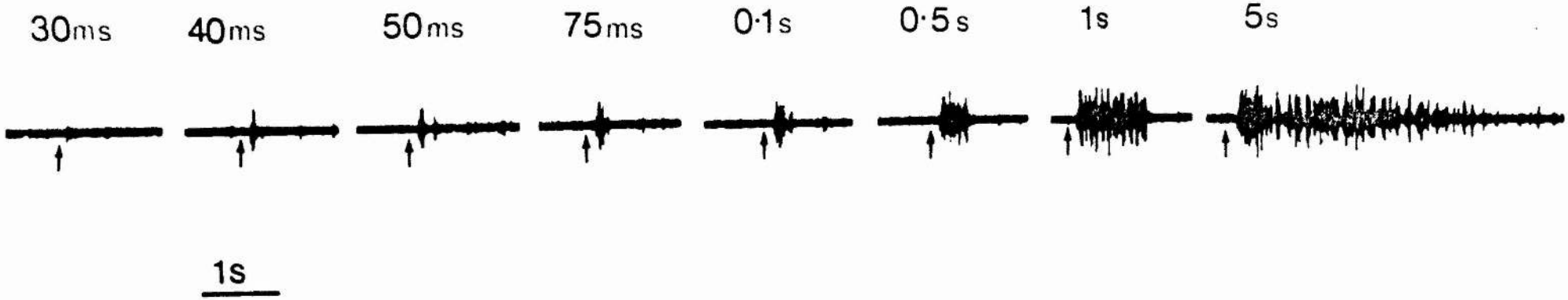

30ms
40ms
50ms
75ms
0·1s
0·5s
1s
5s
1s

FIG. 5.

Graph showing total number of spikes in response to three shadow lengths calculated using spike amplitude discrimination. The values on the X-axis are arbitary values of spike height ranging from betwen 15 (maximum spike height) to 2.5 (just above `noise'). Spikes were classified on amplitude recorded into six bands. Vertical bars indicate S.D. N = 25 for each shadow length.

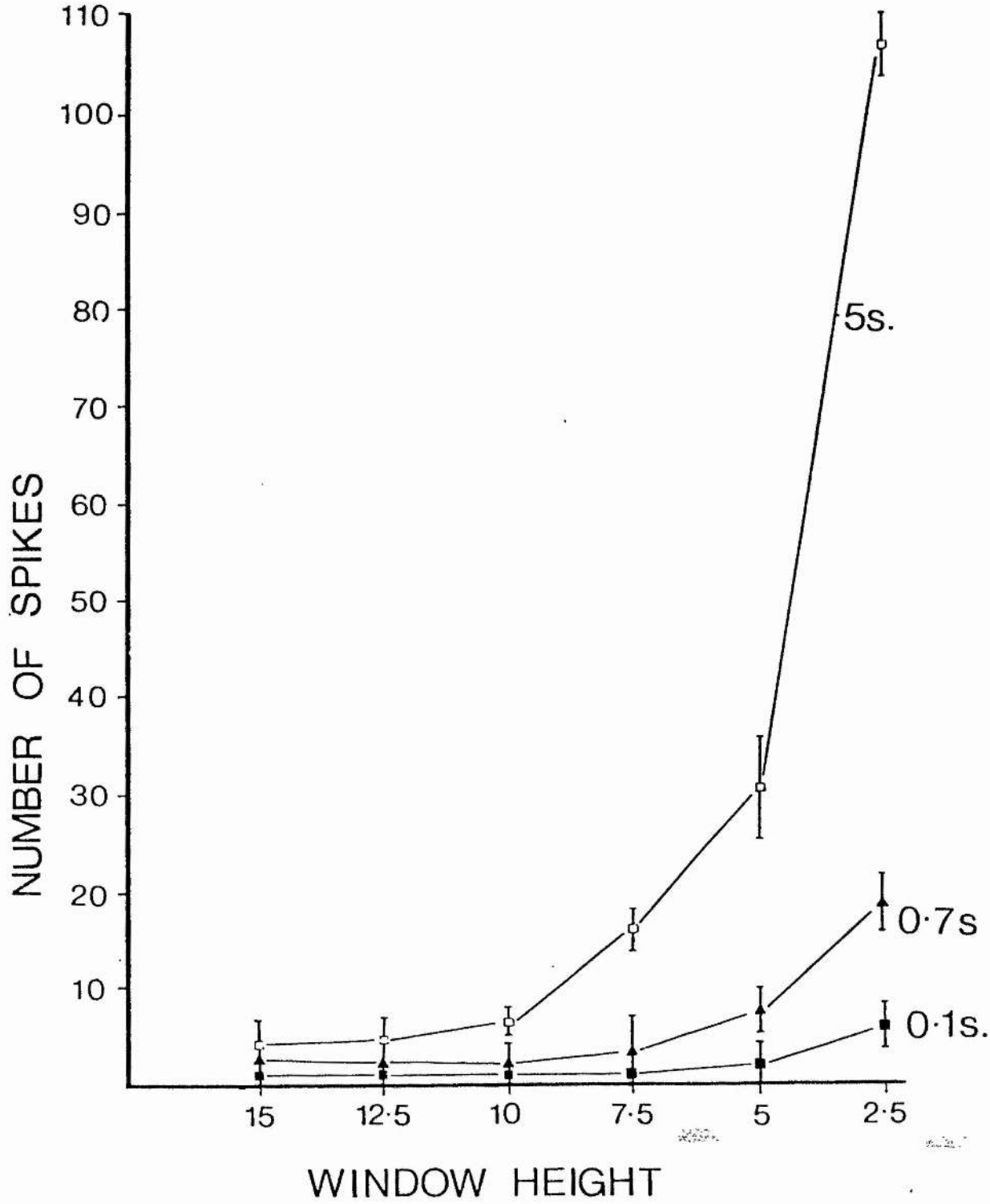
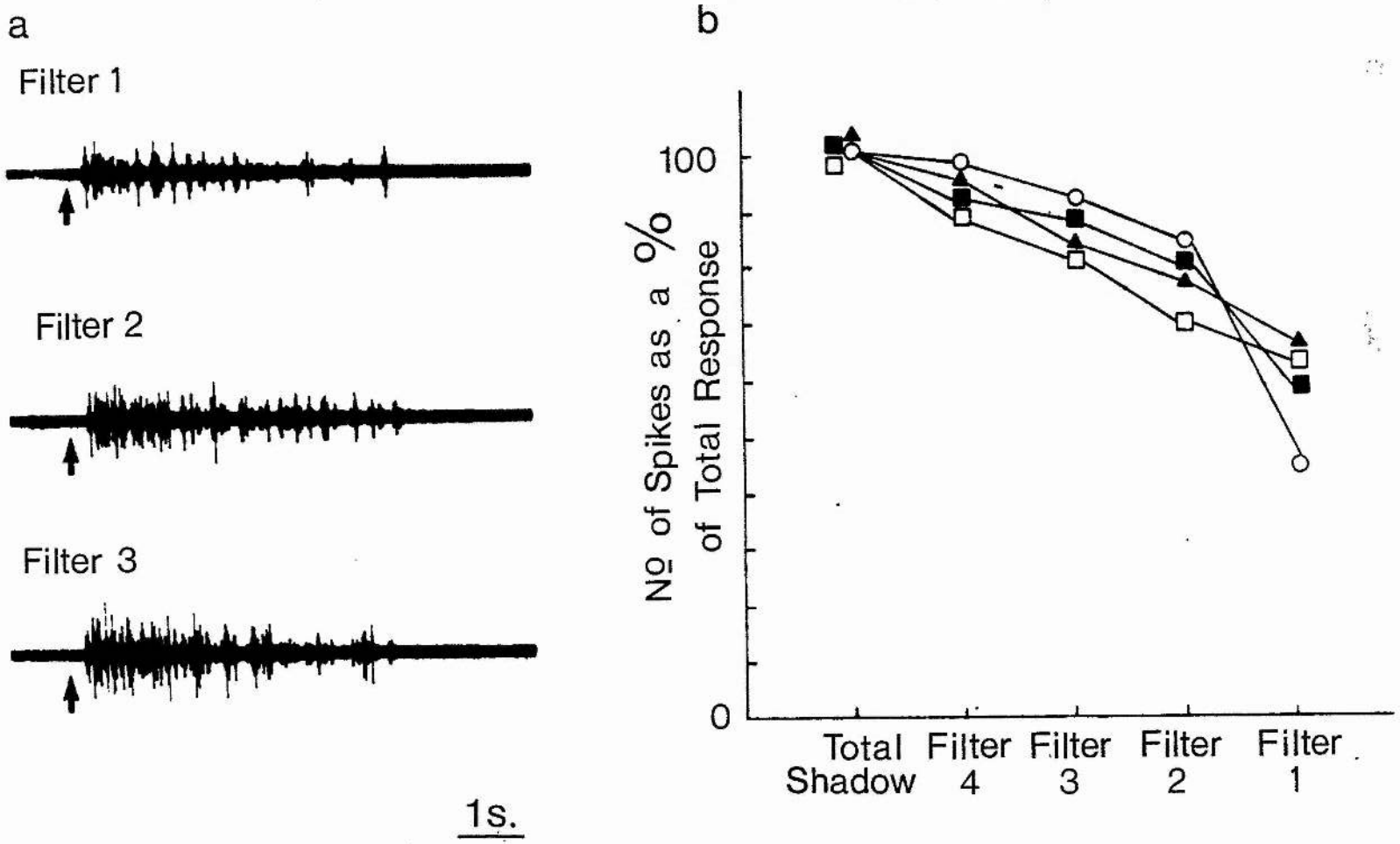

110
100
90
80
70
60
50
40
30
20
10
NUMBER OF SPIKES
5s.
0·7s
0·1s.
15
12·5
10
7·5
5
2·5
WINDOW HEIGHT

FIG. 6.

a) Extracellular responses recorded to increased shadow density. The filter number corresponds to the number of identical neutral density filters inserted to produce the shadow. Arrow indicates point of shadow stimulus. b) Number of spikes within each response to a shadow of increasing density. This was produced by increasing the number of neutral density filters used and plotted as a percentage of the number of spikes present in a total shadow response. The four plots represent typical responses chosen from over 15 similar preparations.


a
Filter 1
Filter 2
Filter 3
1s.
b
100
0
No of Spikes as a % of Total Response
Total Shadow
Filter 4
Filter 3
Filter 2
Filter 1

increasing density were obtained by placing increasing numbers of neutral density filters between the light source and the fibre optic cable. The level of shadow was not quantified as the experiment was intended only to examine the animals ability to discriminate the relative amounts of shadow produced.

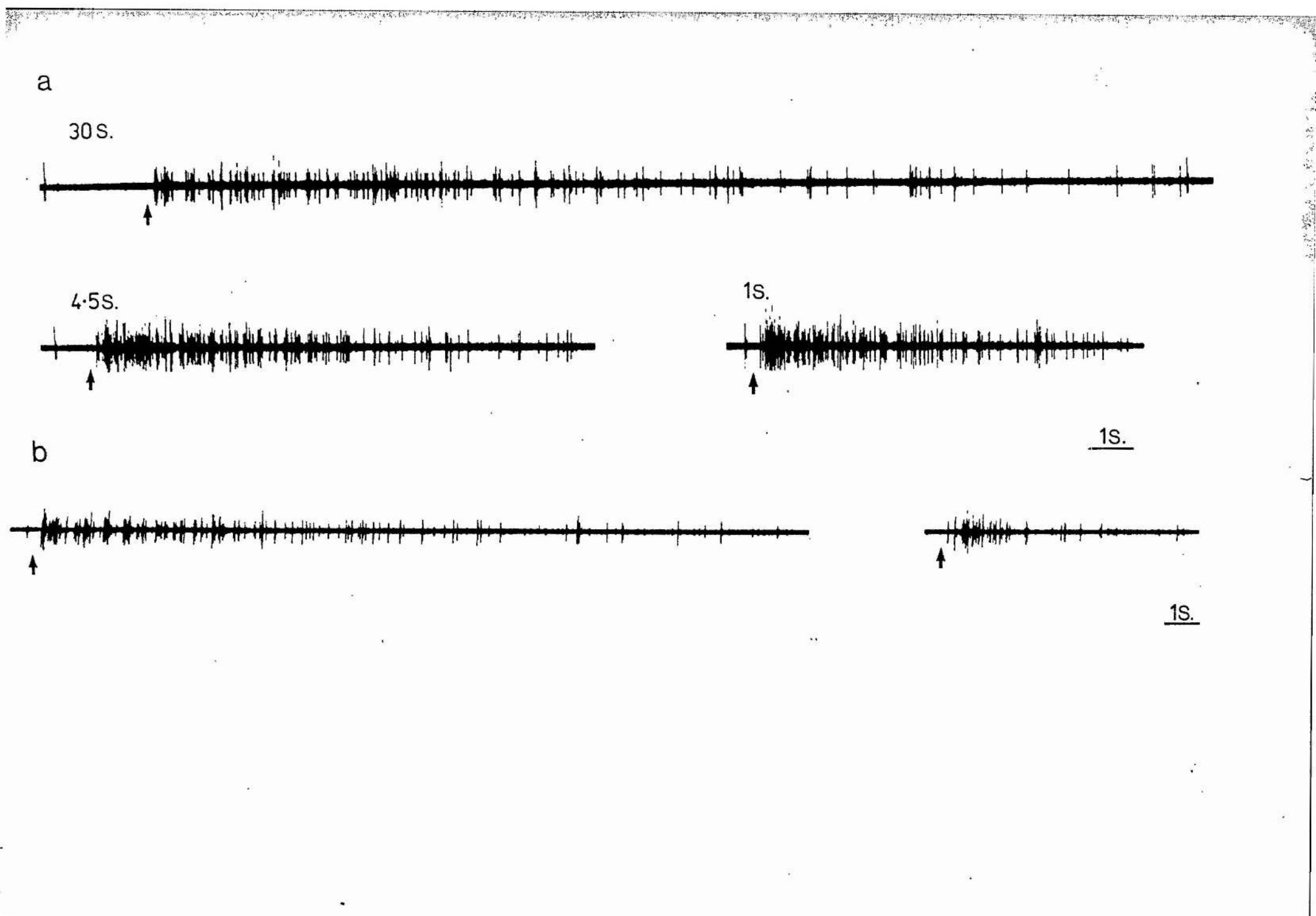
Each shadow density produced a spike train (Fig.6) of single unit activity. The total number and amplitude of units within each 5 second response increased as the filter density and hence depth of shadow increased. At the higher shadow densities, large units, amplitude 40-50μv were evident within the response. The brittlestars were shown to be able to discriminate the amount of shadow produced on the surface of the arm.

Rate Of Change Of Shadow.

The responses of the brittlestars to relative rates of change in shadow density were examined. The light spot 1.5mm in diameter was extinguished by a motor driven rheostat to produce a total shadow. The speed of the motor was varied to extinguish the light at three different rates, in 30 seconds, in 4.5 seconds and in 1 second (Fig.7a). In all cases spikes were recordable from the R.N.C. as soon as there was a decrease in light intensity and continued after the light was totally extinguished. The faster the light was extinguished the greater was the recorded response in terms of total

FIG. 7.

a) Extracellular recordings from the R.N.C. in response to different rates of change of shadow. Total shadows were produced by a uniform reduction of illumination to zero in 30 seconds, 4.5 seconds and 1 second. Note initial bursts of spikes at onset of shadow in each response. (arrow).
b) Extracellular response recorded to a shadow produced over a 30 second period. The first arrow indicates the initial reduction in the light level. The reduction in light was then halted after 15 seconds (second arrow) and the animal allowed to acclimitise to the new lower light level for 15 minutes. The light was then reduced again at the same rate (third arrow) to produce a total shadow. Note the burst of spikes at the point where the shadow is again produced after the 15 minute period of habituation at the lower light level.


a
30 S.
4·5S.
1S.
b
1S.
1S.

number and amplitude of the recorded spikes. The initial high frequency burst of units within the pattern became more complex with increasing rate of shadow produced and this was recorded at the onset of the decrease in light intensity, and not at the point when the light was finally extinguished. Reducing the illumination in this manner inevitably resulted in the production of different wavelengths of light and hence colours before a shadow was finally produced. Stubbs (1982a) has demonstrated the ability of this brittlestar to discriminate various wavelengths of light. The animals in the present experiments may have responded to the different colour changes as well as the rate of shadow production. However as the experiments were concerned with the relative rates of reduction in the light levels and the colour changes were the same from one experiment to another, it was considered that this additional factor did not detract from the overall results. The complex burst of activity at the onset of the shadow indicated the presence of a phasic component, whilst the spikes recorded throughout the reduction in illumination was evidence of a tonic component within the response. An investigation was carried out to determine whether the animals were responding to the initial reduction in the light intensity. Experiments were carried out where a light, extinguished over a 30 second period was halted after 15 seconds, and the animal allowed to adapt to the new lower light level for 15 minutes. The light was then extinguished at the same rate to produce a shadow (Fig.7b). A complex high frequency burst of single unit

activity was recorded at the onset of the 30 second reduction in light. A similar burst of activity, but not as complex, was again recorded at the continuation of the light reduction and preceding the adaptation to the new lower ambient light level. The brittlestars were thus shown to be responding to the initial reduction in light intensity (shadow), irrespective of the ambient light levels and any previous response. O.ophiura discriminated between the relative rates of an increase in shadow density produced on the surface of the animal.

Light `ON' Response.

The ability of O.ophiura to detect increases in the ambient light level the light `ON' response was investigated. Increasing numbers of distal arm segments were illuminated via fibre optic cable for up to 10 seconds after a period of 1 hour in total darkness. There was no recordable activity in response to the illumination of less than 5 arm segments. However above this number a small number of very small amplitude units were discernible (15μv), but large spikes similar to those found in the work of Stubbs (1982a) were never recorded. Because Stubbs did not carry out his experiments in total darkness, it is possible he was recording a response to a reduction in the ambient light levels he was not aware of. Increasing the area of the arm illuminated resulted in a corresponding increase in the number of these small recorded spikes. In all cases

FIG. 8.

Light 'ON' response. Response to illumination of ten segments of the arm tip. A small burst of discernable spikes are evident (arrow). Illumination of fewer segments lead to either a smaller response or no response.

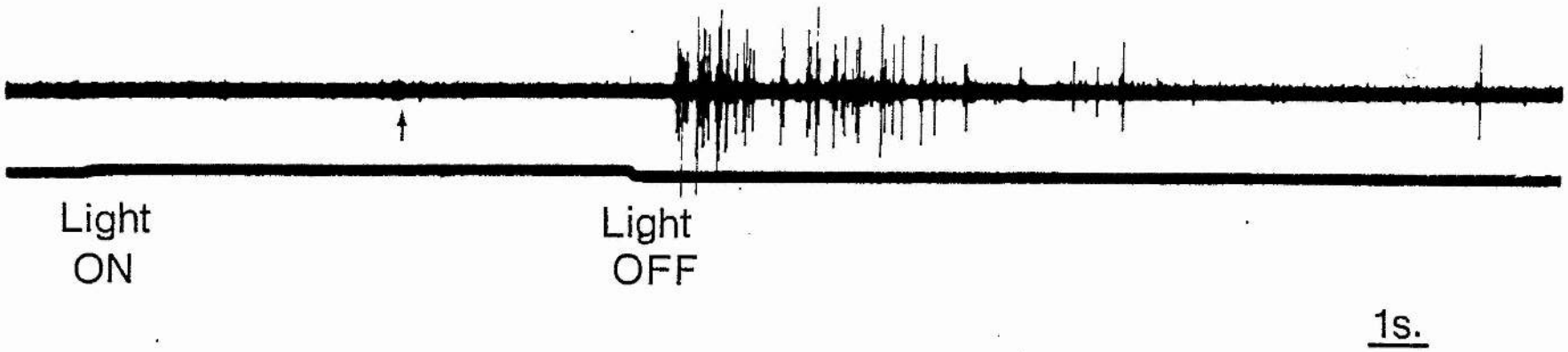
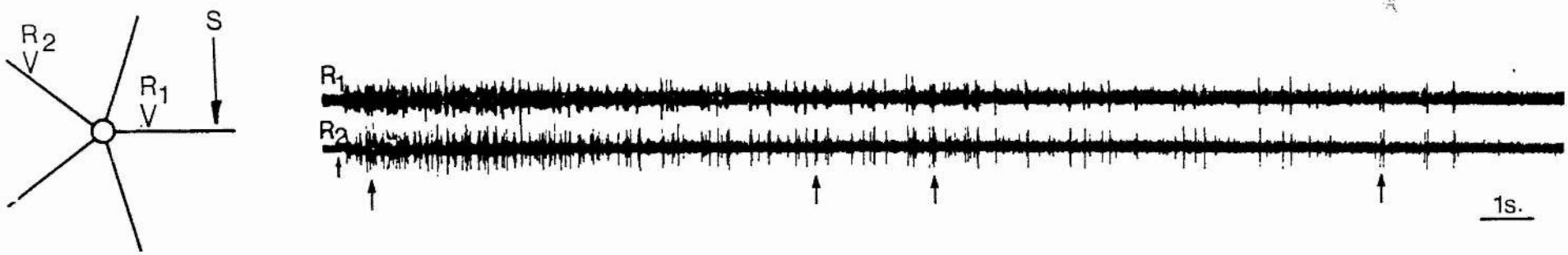

Light
ON
Light
OFF
1s.

FIG. 9.

Extracellular responses to a shadow stimulus recorded simultaneously from the R.N.C. of different arms. (arrow). The shadow response is conducted to the other arm in a substantially unaltered form. Arrows indicate units or groups of units detected at both recording sites.


R2
S
R1
R1
R2
1s.

a spike train of potentials was recorded after the 10 second illumination when a shadow was produced. (Fig. 8).

Multiple Electrode Recordings.

Simultaneous recordings with two electrodes at different positions on the R.N.C. indicated that the pattern of response to a shadow stimulus was conducted to other parts of the animal in a substantially unaltered form (Fig.9). Groups of similar units (approx. 35μv) within the pattern were detected at both recording sites in response to a shadow on the tip of an arm. These results were similar to those of Stubbs (1982a) who carried out a more detailed investigation on the way the photic response was conducted through the radial nerve cord.

Behavioural Observations.

Two groups of animals were maintained in sand bottomed circulation tanks under different light regimes. One group was dark adapted for a period of several weeks, the only source of illumination being a dark red photographic lamp. Stubbs (1982a) has shown that Ophiura ophiura is least sensitive to light of this wavelength. The second group was maintained at ambient light levels which varied from daylight to fluorescent

strip lighting. In both cases the effect of a shadow passing across the surface of the animal was examined and the behaviour of the whole animal noted.

Dark adapted animals were initially illuminated for five seconds with a tungsten filament torch which was extinguished to produce the shadow stimulus. This was an unnatural stimuli to present to the animals, but the behaviour of the brittlestars to these stimuli in dark adapted conditions were required to compare with the neurophysiological experiments. A similar stimulus was presented to light adapted animals and in addition a shadow stimulus was produced by passing a 15cm x 15cm card between the animal and the general direction of illumination by the ambient light source.

Each stimulus was presented to animals that were performing any type of movement, e.g. strong locomotary, arm coiling, arm waving, or burrowing behaviour with the tube feet. The response of all light adapted animals to a shadow produced by the card was an immediate "freezing" of movement whatever their position or type of behaviour being performed at the onset of the shadow stimulus (Table 1). Those illuminated by a light spot which was then extinguished also responded by freezing but the response was not so consistent as it was to a reduction in the ambient illumination with the aid of a card. In this case only 74% of the animals froze to the onset of the shadow.

TABLE 1: Responses to photic stimulation, of two groups of brittlestars maintained at different light regimes

STIMULUS	DARK ADAPTED ANIMALS		LIGHT ADAPTED ANIMALS	
	% of Animals 'Freezing'	No Response	% Animals 'Freezing'	No Response
Shadow produced by the movement of a 15cm x 15cm card between animal and ambient light source. N = 50	-	-	100	0
Shadow produced by illuminating the animals for 5 seconds with a tungsten filament torch and then extinguishing the light. N = 50	68	32	74	26
Shadow produced by illuminating the animals for 1 second with a tungsten filament torch and then extinguishing the light. N = 50	62	38	68	32
Illumination of the animals with the tungsten filament torch for up to 3 minutes. N = 50	0	100	-	-

Dark adapted animals undergoing similar movements were only stimulated with a shadow after illumination for 1 second. The animals again generally responded to this stimulus by freezing their movements but once again this response was not so consistent as the response of light adapted animals when the ambient light level was reduced to produce a shadow.

In all cases where a freezing of the animal movements occurred in response to a shadow stimulus, movement recommenced between 15-24sec. later.

The light 'ON' component of the stimulus used on the dark adapted animals (e.g. when the torch was initally turned on for 1 second) was not involved in the freezing of the brittlestars movements. Fifty seperate animals showing movement were illuminated for up to 3 minutes and the characteristic freezing response did not occur at any point during the illumination but did so when the light was finally extinguished. (Table 1). Brittlestars starved of food for two weeks immediately respond to simple chemical cues and food items e.g. human skin secretions and ground up Mytilus edulis tissue, introduced into the water column by exhibiting arm coiling movements and a strong attraction towards the source of the stimulus. Under these circumstances in both dark and light adapted animals, shadow stimuli of any form had no effect on the movement of the animal. The freezing of the animal to the onset of a shadow

stimulus was not exhibited.

DISCUSSION

The photosensitivity of the echinoderms has in the past been construed from changes in the behaviour of either the whole or parts of the animal (see Yoshida 1966 for review). Apart from an isolated observation by Hartline et al (1952) and an experiment by Takahashi (1964) on the isolated nerve cord of Diadema setosum, electrophysiological evidence for the existence and degree of photosensitivity has been lacking.

However, Stubbs (1982a) using suction electrodes attached to the R.N.C. showed that the species Ophiura ophiura is maximally sensitive to blue green light of the wavelengths found in shallow marine habitats (470-500nm) and light levels (10^{-5} uW/m^2) that are comparable with those ambient in their natural habitat. Millott and Yoshida (1956) demonstrated, using the withdrawal of the podia, that Psammechinus was most sensitive to wavelengths of between 440-560nm and later Yoshida and Millott (1960) using light spots on the radial nerve cord demonstrated that in Diadema the maximum sensitivity to wavelengths of light lies between 455-460nm.

This study has described in detail the shadow response of Ophiura ophiura and shown the animal to be highly sensitive to reductions in the ambient light levels and to a much lesser extent to an increase in illumination. Stubbs (1982b) demonstrated that the greatest sensitivity to photic stimulation lay within the region of the arm tips. This is of adaptive significance considering its characteristic posture, buried in the sand with just the tips of the arms protruding.

The present neurophysiological studies show that O.ophiura is most responsive to a fast moving, dense shadow passing across the surface of the arm. The shadow response has great adaptive significance for the brittlestar in its natural environment. Ophiura ophiura is extensively preyed upon around the coasts of Britain by gadoids and flatfish, two groups of primarily visual feeders (Stevens 1930). The detection of a rapid moving shadow may well be critical to the brittlestar and the density of the shadow may well relate to the closeness of the predator. If all motion ceases at the onset of a deep, fast moving shadow, the brittlestar may well escape detection by a visually orientated predator. Millott (1975) described the movement of spines towards the source of a shadow stimuli and suggested that in Diadema the shadow reaction may also assume an important role in the survival of this animal as a defence mechanism against predators. Crozier (1914, 1915) also showed in Holothuria captiva, H.capita and H.rathbuni, that shaded

animals retract the tentacles, close the brim and cloacal sphincter and show a general contraction of the body wall, which infers a possible advantage to the animal in avoiding predation. More recently Bonham and Held (1963) have demonstrated similar behaviour in other holothurians.

Ophiura ophiura is able to detect increases in illumination or light 'ON' stimuli, but the response requires stimulation of a large part of the surface of the arm and the threshold is higher than that required to produce a shadow response. Increases in illumination may not be associated with a threat to the animal and information regarding increases in light levels may not be conducted through the large, and hence rapidly conducting neurons but through some of the larger number of smaller neurons whose spike potentials either are too small to be recorded or lost in the background noise. Millott and Yoshida (1959) demonstrated in Diadema antillarum the threshold for 'ON' responses was higher than that required to produce a shadow response. Millott (1955, 1956) also demonstrated both 'ON' and 'OFF' responses in the podia of Lytechinus variegatus and once again the animals were more sensitive to shadow than an increase in illumination.

Specialized photoreceptors are rare within the echinoderms. Penn and Alexander (1980) described the structure of an optic cushion in Nepanthia belcheri: a pigmented sense organ situated on the oral surface of the

arm below the terminal tentacle. This was similar to the optic cushions reported in other asteroids (Eakin 1963). In general there appears to be no special sensory organs associated with photosensitivity. It is likely that in brittlestars there is a dermal light sense as described by Millott (1975) in echinoids. Marked responses to shadowing are characteristic of animals with a dermal light sense. There are two possible explanations for the immediate cessation of recordable activity within the nervous system to the reillumination of the arm tip after the shadow stimulus. These two alternatives depend on the biochemical mechanism present at the site of transduction. It is reasonable to presume that a photically activated pigment is present, as this is the case in the majority of invertebrate photoreceptors (Eakin 1972). Alterations to the stucture of this photopigment may cause a permeability change in the receptor cell membrane. A photically activated pigment may cause a permanent hyperpolarization of the membrane which depolarizes to a threshold when a shadow is cast. The alternative is a photically inactivated pigment which becomes activated and causes a depolarization to threshold at the onset of shadow. The inhibitory effect of light has been suggested by Hess (1915) working on the rotary spines of Centrostephanus longispinus and more recently by Millott and Yoshida (1960) and Yoshida (1962) working on two allied species of echinoids. However, the ability to record extracellular potentials from the R.N.C. to an increase in illumination in O.ophiura and its negative phototactic behaviour are difficult to

explain if light has an inhibitory effect on the photoreceptive system. The transduction mechanism in the brittlestars photoreceptive system is still not understood and at present no physiological evidence of the inhibition by light has been produced from individual sensory neurones.

The important observation that the shadow response, although always recorded from the nervous system, is overridden behaviourally by the presence of food suggests the presence of a behavioural heirarchy directly dependant upon the physiological and nutritional state of a particular animal. This has particular significance when experiments on the sensory discriminatory powers of echinoderms are based solely on behaviour changes in the animals. It is important that the whole range of sensory modalities present are absolutely defined during experimentation to avoid many of the inconsistencies that occur under such conditions.

CHAPTER 2

CHEMOSENSITIVITY

INTRODUCTION

Romanes, (1883) showed over a century ago that the starfish Asterias rubens was attracted to fresh crabmeat. Sloan and Campbell (1982) have recently reviewed the substantial literature since then on chemoreception in the Echinodermata, some of which however is almost anecdotal. Recent work by Zafiriou (1972), Valentincic (1973, 1979) and Reimer and Reimer (1975) has attempted to characterize what particular molecular structures act as most effective stimuli. Amino acids and their derivatives have been shown to be effective in eliciting changes particularly in the feeding behaviour of a number of echinoderms (Valentincic 1979). This chapter describes, using extracellular recordings from the radial nerve cord the responses of O. ophiura to a range of amino acids. The sensitivity to the amino acids and the thresholds obtained are compared with those from parallel studies where the behaviour of unfettered animals was observed in a series of laboratory tank experiments. The feeding behaviour of the brittlestars and the ability to detect prey and predator items by distance chemoreception are also described.

MATERIALS AND METHODS.

Neurophysiological Preparations.

Single arm preparations were dissected as described in the previous chapter to reveal the R.N.C. The arm was then partially immobilized oral side uppermost in a perspex clamp, leaving only the distal 15 segments of the arm free. The clamped arm was then placed in a two chambered dish (14 x 25 x 2.5cm) containing filtered seawater. The dish was divided into two equal portions by a partitioning perspex barrier in which a 5mm. diameter hole had been bored. The tip of the arm was placed through this hole and the gap sealed with Vaseline. This ensured that the tip of the arm was physically isolated from the rest and that only this portion would be stimulated. It also ensured that the recording electrode would not be displaced by the flow of seawater. Filtered seawater at $4^{o}C$ flowed from a constant pressure reservoir via polythene tubing over the arm tip at a rate of $15cm^{3}$ $minute^{-1}$. The animal was allowed to acclimatise in total darkness for 1 hour before each experiment. This was to remove all the other stimulatory factors except the chemical stimulus under investigation.

Experimental Procedure.

Serial dilutions of each amino acid and Betaine, (Sigma Chemicals), in seawater were prepared from a 0.5×10^{-2}M stock solution. The amino acids used were L-Leucine, L-Lysine, L-Cysteine, L-Serine, L-Alanine, L-Proline, L-Valine, N-acetyl-L-Cysteine, Malic Acid and Lactic Acid. These were chosen because they had been used by other workers. (Valentincic 1979, Reimer and Reimer 1975). Each solution was made up using filtered seawater an hour before each experiment and kept at a constant 4^{o}C. temperature. Beginning with the lowest concentration, $0.5cm.^{-3}$ aliquots of each concentration of amino acid solution was injected at a constant rate into an intermediate reservoir, where they were completely mixed with the filtered seawater and passed over the arm tip. Each aliquot was diluted by a factor of 20 before reaching the isolated arm tip. A control injection of an $0.5cm.^{-3}$ aliquot of filtered seawater was used prior to each addition of the various amino acid concentrations. A period of 20 minutes untreated flow was allowed between the addition of each concentration to prevent possible habituation of the response. Recordings of each response were made using the methods described in the previous chapter.

Naturally occurring amino acids in seawater were not quantified since the experiments were designed to test the ability of the brittlestar to perceive changes in concentration. All seawater used was obtained freshly pumped from the sea and filtered, however, as a further control, artificial seawater Instant Ocean, (Aquarium Systems, Ohio), was also used both untreated and with various amino acids. The thresholds of response obtained were identical with those from natural seawater.

Behaviour.

Experiments were carried out on whole animals using behavioural criteria to determine the minimum detectable concentration of solutions of the same amino acids which were used in the neurophysiological experiments. A single animal was placed in each of four separate compartments formed by dividing longitudinally a perspex tank 11.5 x 300 x 3.5cm. and a constant water flow of $15cm.^{3}$ $min.^{-1}$ was maintained through each compartment by a set of valves at each inflow. The water was filtered before entering the tank and turbulence was reduced by a fine plankton net barrier. Each animal was handled using rubber gloves and plastic forceps and placed on a predetermined spot where the concentration of the amino acid, after dilution effects would correspond to that used in the neurophysiological experiments. Animals were allowed to acclimatise in the tank for 1 hour before each experiment. The stimulus was presented by injecting, one

at a time, 0.5cm.^{3} of each separate amino acid and Betaine concentration prepared by serial dilution into the inflow of each compartment. The solution was mixed well in the turbulent area before passing through the plankton net and down over the animal. The animals were quiescent for at least 5 minutes before the stimulus was presented. The behaviour was then monitored for 3 minutes after each addition, and activity (locomotion, arm coiling, arm waving) was scored as a positive detection of the amino acid. 20 minutes was allowed between each stimulus to avoid habituation. All experiments were carried out using a red photographic lamp for illumination. This reduced the chances of stimulating the animals by a shadow which causes cessation of activity in these animals (Moore and Cobb 1985a).

Neurophysiological Responses To Secretions Of Other Fauna.

In these experiments a larger intermediate chamber, diameter 10cm, replaced that used to determine the minimum threshold concentrations of the amino acids. Species of marine fauna that were collected in the trawl together with the brittlestars were introduced into the chamber to examine the responses of the ophiuroid to any water borne chemicals derived from the animals. The animals used were Asterias rubens and Pagurus bernhardus. The chemosensory ability of the brittlestars to detect other species was tested. After

introduction into the chamber the responses of the ophiuroid were recorded for 2 minutes. Again all experiments were carried out in total darkness and 30 minutes was allowed between each stimulation to prevent habituation.

Behaviour experiments were also carried out on whole animals using the same stimulus test species as in the neurophysiological experiments. Using forceps and rubber gloves each test species was introduced into the sand bottomed circulation tanks containing the ophiuroids. The experiments were carried out under the illumination of a red photographic lamp and the behaviour of each brittlestar was observed for 5 minutes. Five minutes were chosen as this was the time period in which the animals would show a response.

RESULTS

Neurophysiology.

Responses in the form of single unit potentials were recorded from the R.N.C. to each of the 10 amino acids and Betaine tested. Two typical sets of results recorded extracellularly to a range of concentrations of the amino acids L-Leucine and L-Proline are shown in Fig.1 & 2. No recordable activity was evident in response to the seawater control, or at the concentrations of 10^{-15}M, 10^{-14}M. and 10^{-13}M, but the addition of a 10^{-12}M. concentration of L-Leucine solution produced a small discernible burst of spikes, (10-15μv), which was taken as the minimum threshold concentration. The number and size of the spikes increased with increasing amino acid concentration; the largest spikes were between 40-45μv. Similar results were obtained with L-Proline solutions. However no response was recorded from the R.N.C. until the concentration of the solution reached 10^{-10}M. (which was taken to be the minimum threshold concentration for this amino acid). Again the size and number of the recorded spikes increased with concentration. In some preparations where the concentrations were gradually increased to levels above 10^{-7}M, a recordable response did not occur for all concentrations above this level. When these high levels of amino acids were applied initially to a fresh preparation a similar recordable response was consistently obtained. In preparations that failed to

FIG. 1.

Extracellular recordings from the R.N.C. in response to the addition of different concentrations of the amino acid L-Leucine to the tip of the brittlestar arm. (SW) is the seawater control. All concentrations given are x2M.

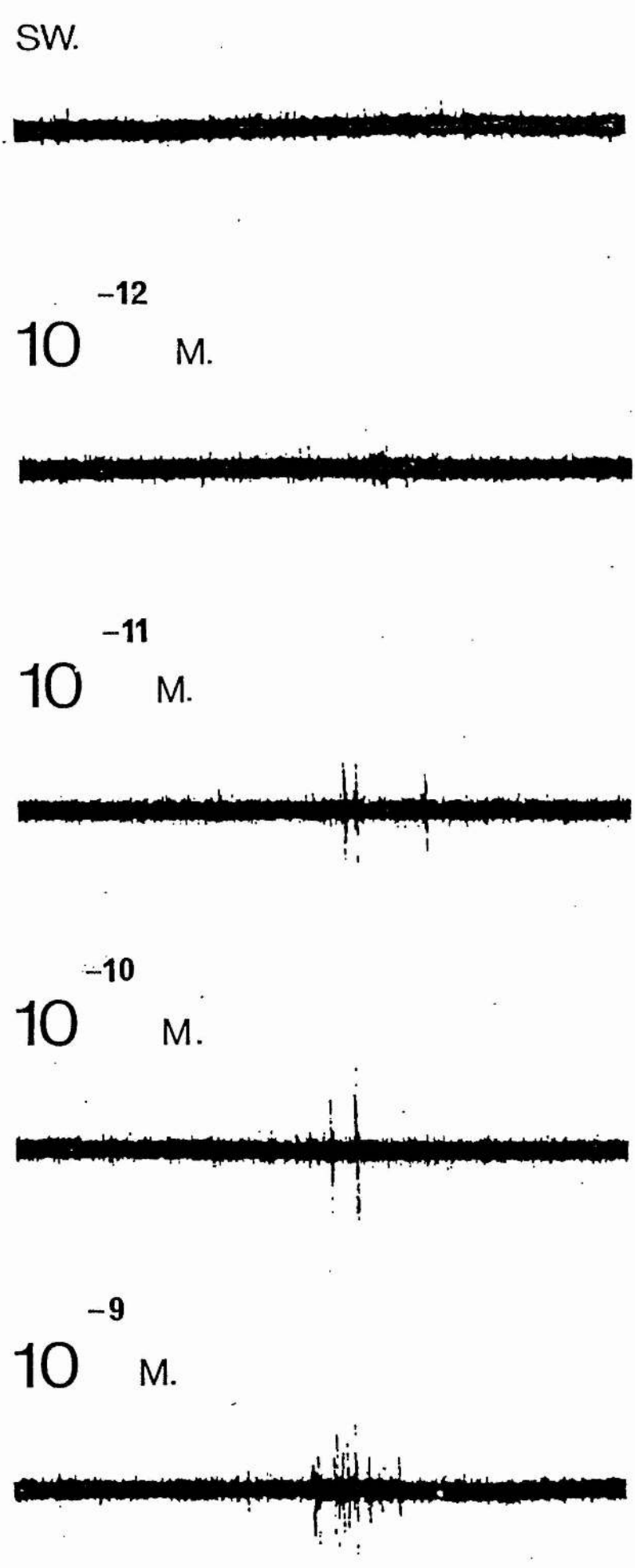
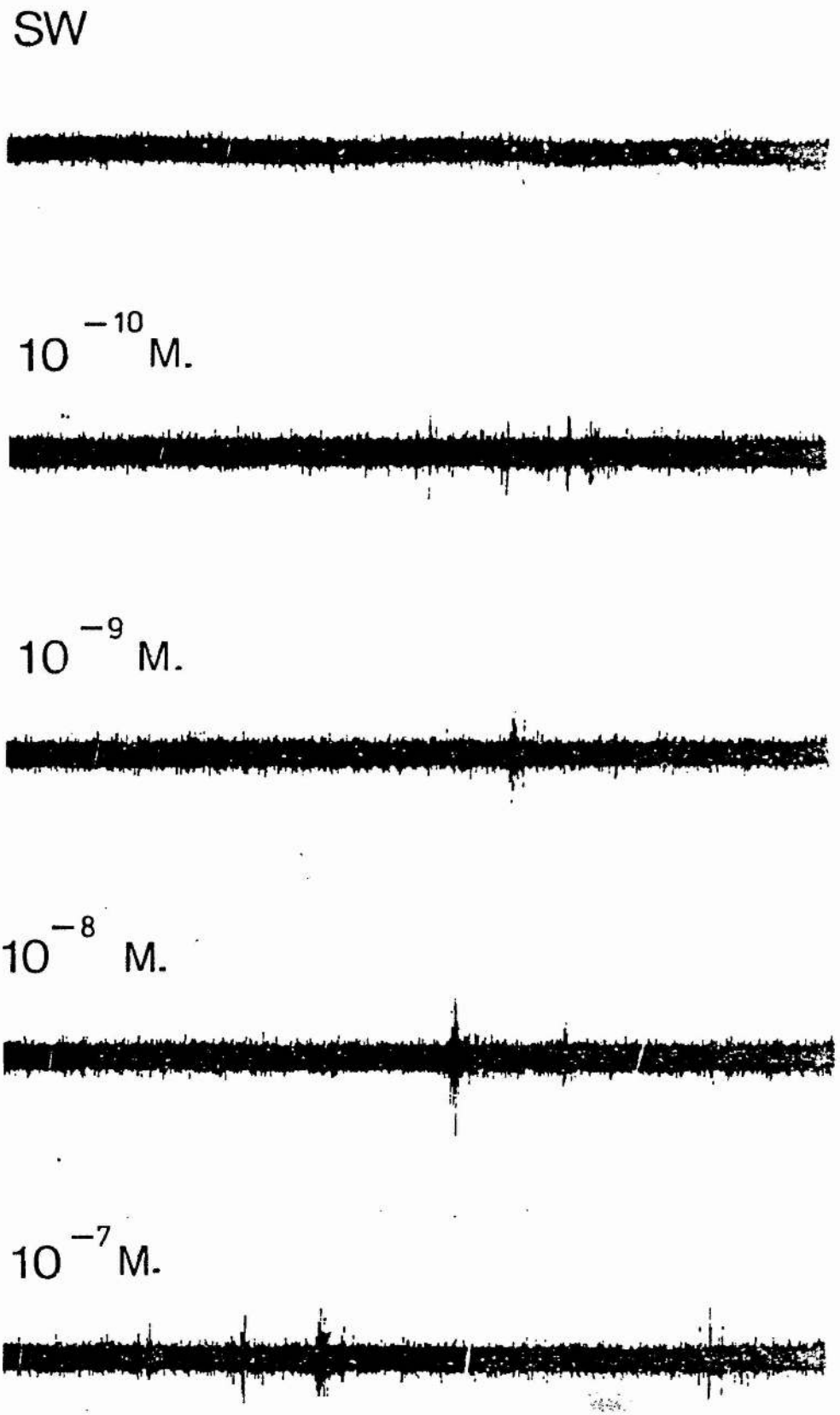

SW.
10^{-12} M.
10^{-11} M.
10^{-10} M.
10^{-9} M.
1 sec.

FIG. 2.

Extracellular recordings from the R.N.C. in response to the addition of different concentrations of the amino acid L-Proline to the arm tip. The seawater is the control (SW). All concentrations given x2M.


SW
10 −10 M.
10 −9 M.
10 −8 M.
10 −7 M.

show responses to high levels of amino acids, a period of washing in plain seawater for an hour restored excitability. The number, size and pattern of spikes recorded differed between each of the 10 amino acids and Betaine used in this study, but no effort was made to relate a type of response to particular molecular characteristics or to concentration. The minimum concentration of each amino acid that elicited consistent recordable activity in the radial nerve cord are shown in Table 1. The amounts detectable are small and represent the ability of the ophiuroid to detect amino acids at levels of parts per billion. Extracellular recordings of the ophiuroids response to foodstuffs were also recorded. Fig.3 shows recordings of the feeding response induced in a single arm preparation by Mytilus edulis tissue. The response involved the coiling of the arm tip laterally, which is characteristic of this species of ophiuroid in the presence of a food stimulus and is desribed in greater detail on page 41.

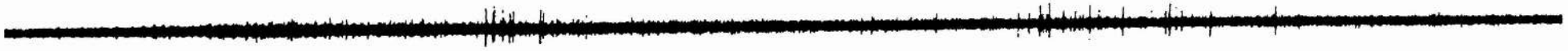
A significant result was obtained when recording simultaneously from two different parts of the R.N.C. the responses to stimulation by firstly a single amino acid and secondly a known food item. The response recorded to the flow of a 2×10^{-7} M. solution of L-Lysine over the arm tip was conducted to other parts of the animal in a substantially unaltered form (Fig.4). However, the patterns of responses recorded in the presence of Mytilus edulis extract were very different when recorded from different positions on the R.N.C.

TABLE 1: The minimum concentrations of 10 amino acids and betaine solutions at which consistent neurophysiological records were obtained from the R.N.C. of Ophiura ophiura.

Amino Acid	Minimum Concentration
L-Leucine	2×10^{-12}M
L-Lysine	2×10^{-11}M
L-Cysteine	2×10^{-11}M
N-Acetyl-L-Cysteine	2×10^{-11}M
L-Alanine	2×10^{-11}M
Malic Acid	2×10^{-11}M
Lactic Acid	2×10^{-11}M
Betaine	2×10^{-11}M
L-Valine	2×10^{-11}M
L-Serine	2×10^{-10}M
L-Proline	2×10^{-11}M

FIG. 3.

Extracellular recordings from a single arm performing a characteristic feeding response (arm coiling), induced by the detection of *Mytilus edulis* extract. Extract added before origin of illustrated trace.



1s.

FIG. 4.

Simultaneous extracellular recordings of nervous activity from two different positions on the R.N.C. in response to to the flow of a 2 x 10M. concentration of L-Lysine solution over the tip of an arm. The stimulated tip was physically isolated by a perspex barrier from the rest of the animal. S is the point of stimulation, R_1 and R_2 are the recording electrodes. Arrows indicate where the pattern of spikes recorded are similar between both recording positions.

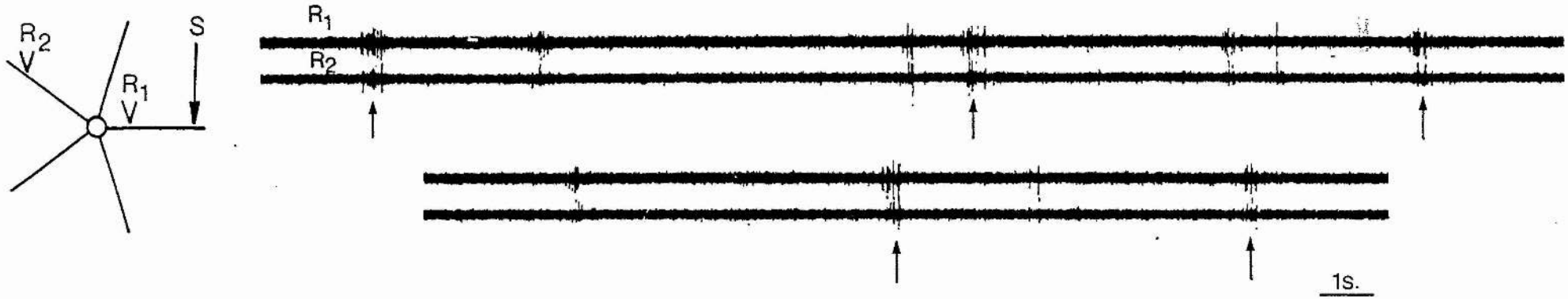

R2
R1
S
R1
R2
1s.

FIG. 5.

Simultaneous extracellular recordings from different positions on the R.N.C. to the addition of Mytilus edulis extract to the flow of filtered seawater over the arm tip. The two traces show very little similarity in pattern at the two recording positions. The arm tip stimulated was isolated from the rest of the animal by a perspex barrier. Point of stimulation (S). Recording electrodes R_1 and R_2 .

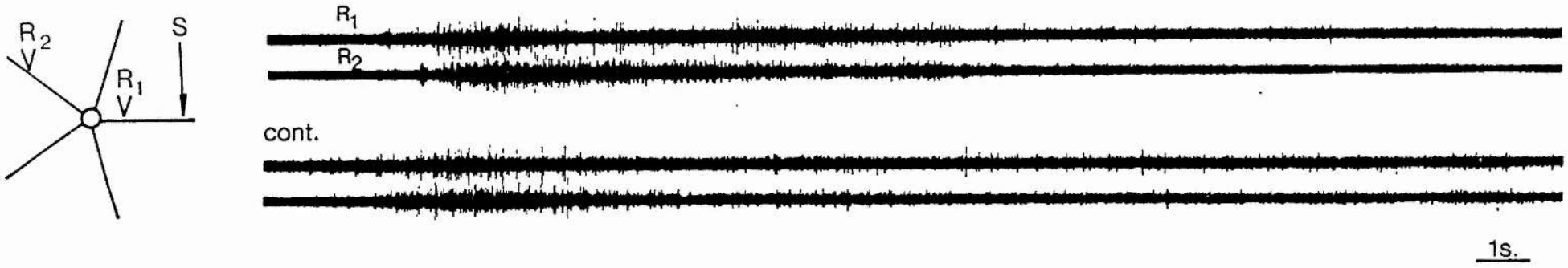
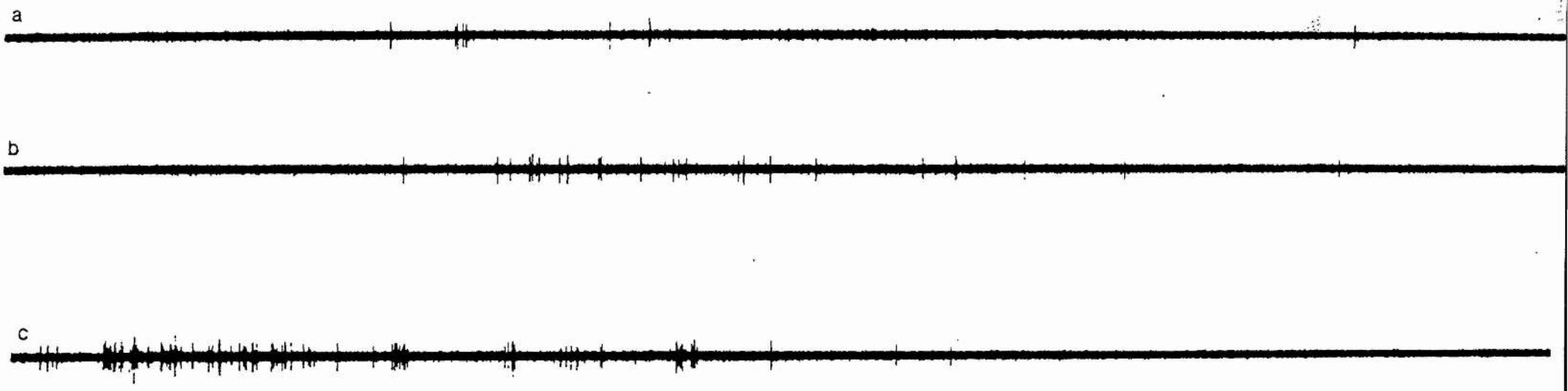

R2
S
R1
R1
R2
cont.
1s.

FIG. 6.

Extracellular recordings from single arm preparations in response to other invertebrates commonly encountered in the brittlestars natural environment, introduced to an intermediate chamber. The water from the chamber flows across the tip of the arm. a) 2.5cm *Asterias rubens*. b) 4.7cm *Asterias rubens*. c) *Pagurus bernhardus*. *Asterias rubens* measured from centre of disc to tip of arm. Note greater response in terms of size and number of spikes recorded to the larger *A.rubens*. Animals introduced before origin of trace.


a
b
c

(Fig.5). The significance of this result is discussed at greater length in the general discussion. (Page 100).

The introduction of A.rubens and P.bernhardus into the water flow passing over the tip of the neurophysiological preparation, produced recordable activity within the R.N.C. Figure 6a,b, and c show the response recorded to two different sized Asterias rubens and Pagurus bernhardus. The size of the response in terms of amplitude and number of recorded potentials increased with the size of the Asterias rubens added to the intermediate chamber. The response to Pagurus bernhardus was similar to that recorded to a shadow stimulus. Spikes ranged up to 40μv in amplitude and there was evidence of discrete bursts of spikes in the response. Detectable activity was also recorded in response to the presence of Pleuronectes platessa of length 3cm and 9cm.

Behaviour.

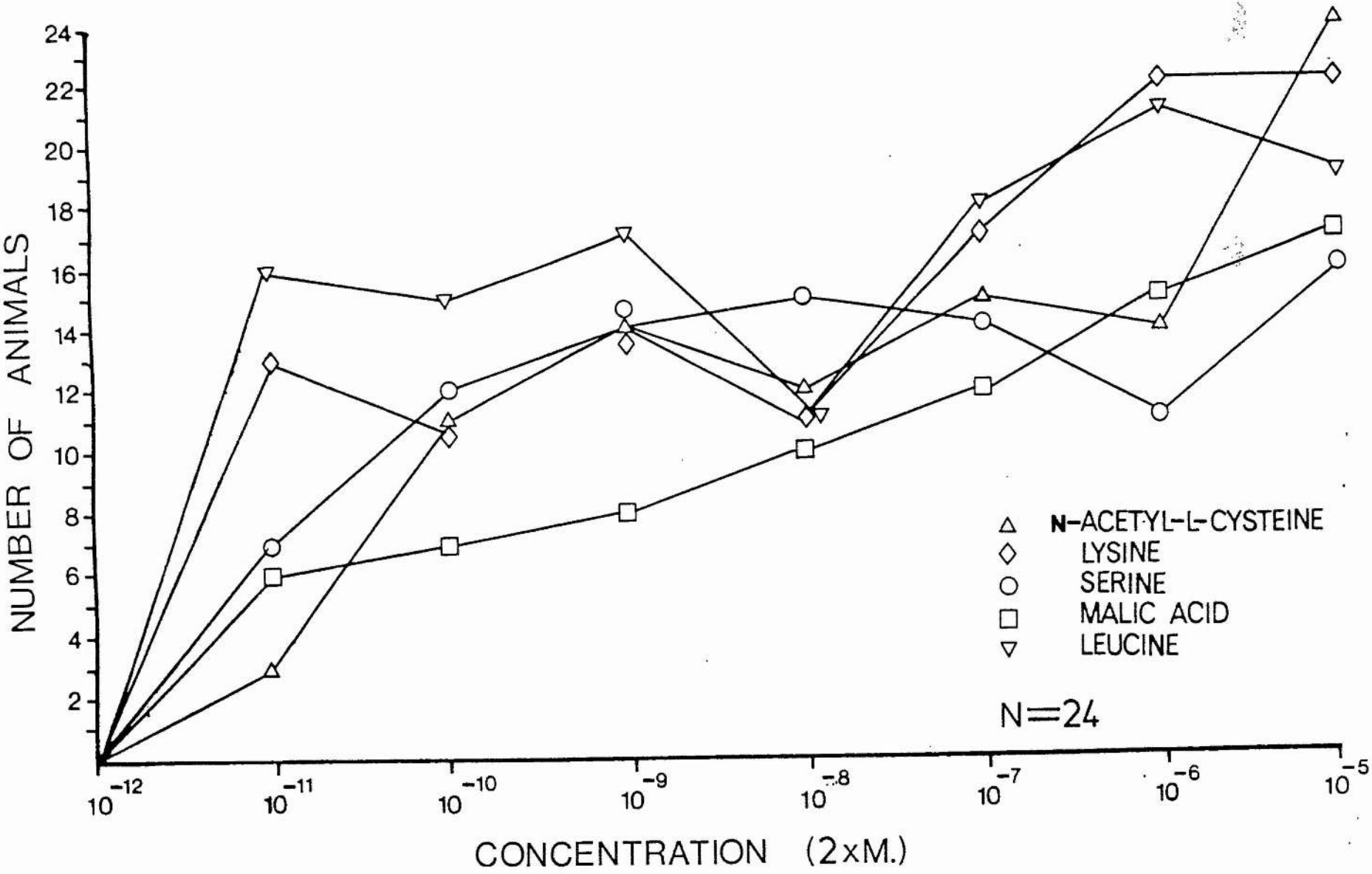
The total number of experimental animals responding to each of the 10 amino acids and Betaine solutions are indicated in Figs.7a & b The responses of the animals to the minimum threshold concentration determined in the neurophysiological experiments for each amino acid showed inconsistencies. In the case of L-Alanine for example none of the experimental animals showed any type of response at a concentration of 2×10^{-11}M, the level

FIG. 7 a and b.

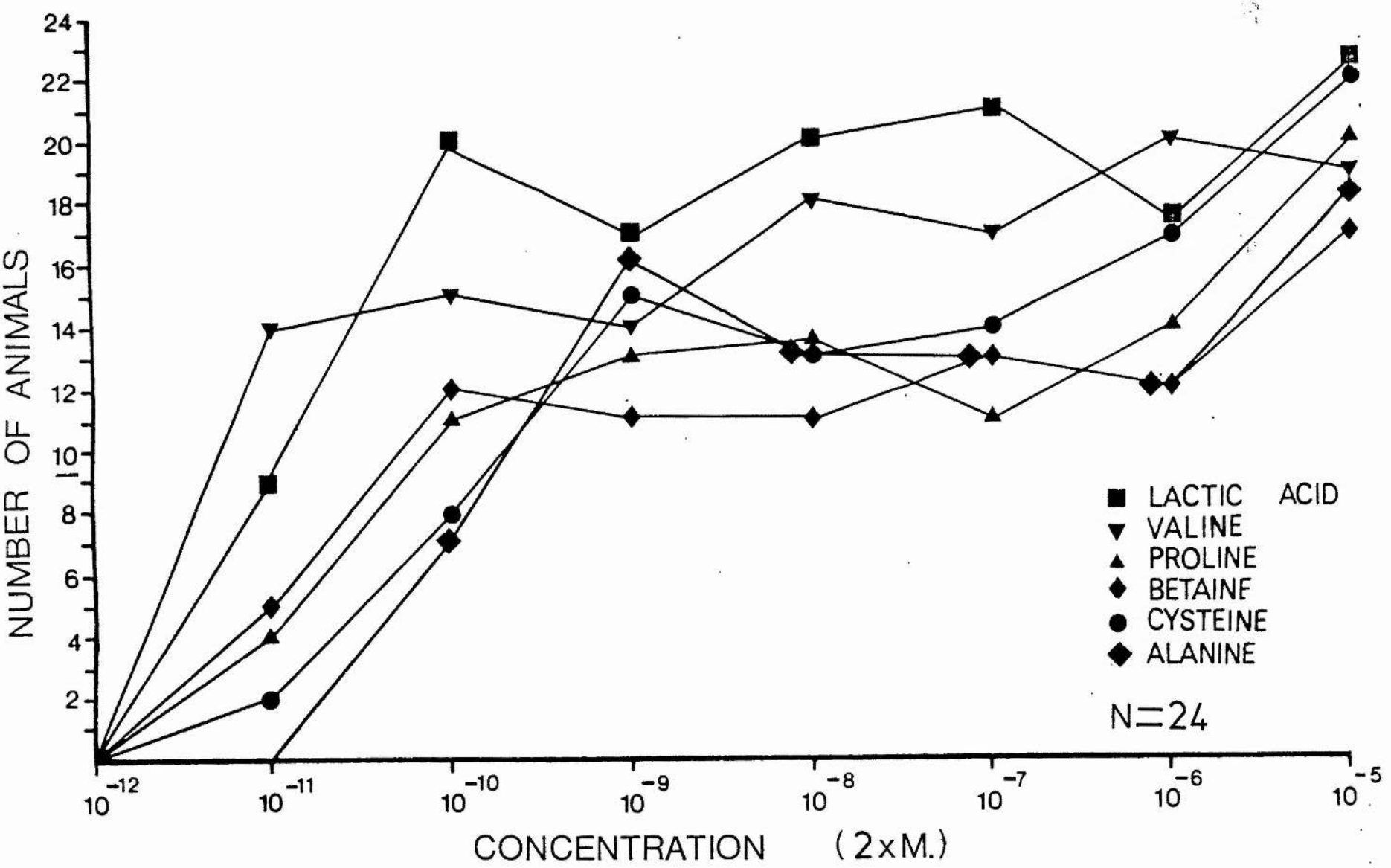
Graphs to illustrate the total numbers of brittlestars showing any type of behavioural responses to different concentrations of amino acid solutions. All concentrations are of the L-isomer.

N = 24.

b.


NUMBER OF ANIMALS
24
22
20
18
16
14
12
10
8
6
4
2
N-ACETYL-L-CYSTEINE
LYSINE
SERINE
MALIC ACID
LEUCINE
N=24
10^{-12}
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
CONCENTRATION (2xM.)

a.


NUMBER OF ANIMALS
24
22
20
18
16
14
12
10
8
6
4
2
LACTIC ACID
VALINE
PROLINE
BETAINE
CYSTEINE
ALANINE
N=24
10^-12
10^-11
10^-10
10^-9
10^-8
10^-7
10^-6
10^-5
CONCENTRATION (2xM.)

which produced consistent neurophysiological responses. Similarly no animal showed any type of behaviour to the minimum threshold concentration determined for the amino acid L-Leucine. A response was only elicited by animals at the higher concentration of 2×10^{-11}M. Consistent activity was recorded from the R.N.C. in response to solutions of the amino acids L-Proline and L-Serine at concentrations of 2×10^{-10} M. However at the whole animal level, behavioural responses were elicited at the lower concentrations of 2×10^{-11}M. The significance of this result is dealt with in greater length in the general discussion, p.98. Ophiura ophiura was attracted to all 10 amino acids and Betaine presented. The behaviour in response consisted of movement towards the source of stimulus, movement of any one of up to five arms in the water column and the characteristic arm coiling response. With all the chemicals investigated there was a noticeable increase in the number of animals showing these types of behaviour with increasing concentration of the solutions. Table 2 indicates the percentage of responding animals that performed the arm coiling response at the different concentrations of the 10 amino acids and Betaine solutions. The arm coiling response was characteristic of O.ophiura in the presence of a food item and was observed prior to the searching behaviour of the animal for the food stimulus. The amino acids most potent at eliciting this feeding behaviour were L-Valine, Lactic acid, Betaine, N-acetyl-L-Cysteine, L-Serine and L-Alanine. However, the results were inconsistent between concentrations and only in the case

TABLE 2: Percentage of responding animals performing the arm coiling response at the different concentrations of the 10 amino acids and betaine solutions. N = 24.

Amino Acid	Concentration of Solution (M.)							
	$2x10^{-12}$	$2x10^{-11}$	$2x10^{-10}$	$2x10^{-9}$	$2x10^{-8}$	$2x10^{-7}$	$2x10^{-6}$	$2x10^{-5}$
L-Leucine	-	17	-	-	-	17	7	11
L-Lysine	-	5	-	-	-	-	-	18
L-Cysteine	-	-	11	-	-	-	11	27
N-Acetyl-L-Cysteine	-	-	9	13	23	7	7	54
L-Alanine	-	-	14	6	23	23	8	22
Malic Acid	-	-	9	-	-	8	-	18
Lactic Acid	-	23	-	7	13	6	32	32
L-Serine	-	-	23	28	15	-	-	50
L-Proline	-	-	9	15	15	36	36	75
L-Valine	-	14	7	21	22	18	20	32
Betaine	-	18	80	12	18	17	57	20

The arm coiling response is characteristic of O.ophiura in the presence of a food item and is observed prior to the searching behaviour of the animal for the food stimulus.

of L-Proline was there a general increase in the percentage of animals exhibiting the arm coiling response with concentration of the amino acid.

The behaviour of the ophiuroids to the presence of Asterias rubens and Pagurus bernhardus in the circulation tanks was very consistent. Brittlestars lying motionless on the substratum remained in this state when approached by both sized Asterias rubens. Contact between the arm tips of A.rubens and the ophiuroids elicited no response nor did the movement of the starfish across the disc of the brittlestar. No response was recorded by any brittlestar to the approach of P.bernhardus. However, any contact between a walking limb or chelae of the hermit crab and any part of the ophiuroid resulted in the immediate movement away from the vicinity of the P.bernhardus and then a sudden cessation of all motion. No movement of any type was recorded from the brittlestars after the introduction of the P.bernhardus except when tactile stimulation occurred.

Observations were carried out on the responses of O.ophiura to the introduction of a food item (Mytilus edulis tissue) into the inflow of a sand bottomed experimental tank. The brittlestars were initially buried in the sand with only the tips of the arms protruding into the water column. In over 35 experiments, the introduction of the M.edulis tissue 15cm away up current of the animal consistently resulted in the following sequence of behaviour:

1. Slight lateral flexure of the visible portion of the arm tip nearest the food item.

2. More rapid flexure of the tip resulting in the coiling of the distal segments.

3. Flexure of the distal half of the arm and the partial emergence from the sand. The other four arms begin to wave in the water column.

4. Animal moved out on to the surface of the sand. All arms were coiling rapidly. The arm closest to the stimulus showed the most vigorous coiling response.

5. Animal moved towards the food item in a "zig-zag" pattern, with the leading arm waving in the water column.

6. On location of the food item the animal coiled the arm around it and the brittlestar lifted itself on to the food.

7. Food was grasped in the mouth and a piece ripped off by the movement of the arm holding the food away from the disc. Food was then ingested.

Experiments were carried out on 40 animals, to observe their responses to a food item placed for different durations close to the brittlestar. Firstly a piece of M.edulis tissue attached to a length of string was lowered to within 2 cms. of the brittlestar and then immediately removed. In all animals the response was only a slight flexure of the arm tips. Secondly the food was again lowered to within 2 cms. of the animals, but then left in this position. In all 40 experiments the animals immediately emerged from the sand and grasped the food without any prior arm waving or arm coiling. This suggests that each phase of the sequence of behavioural response described above was initiated as increasing amounts of the relevant chemical emanating from the food was detected. As certain thresholds of stimulation were exceeded, different phases of the sequence were released. The lowering of the tissue into the water created a mechanical disturbance that was probably detectable by the brittlestars. However, as the rate at which the food was lowered was kept constant measured by a stopwatch, it was considered this would not detract from the overall results.

DISCUSSION.

The utilization of a number of feeding methods in ophiuroids requires that the animals possess an effective chemosensory ability. (For review of ophiuroid feeding methods see Warner 1982). Ophiura ophiura is known to obtain nutrients from suspension feeding (Stubbs and Cobb 1982) and scavenging, (Nagabushanan and Colman 1959) and predation. Peripheral cellular tissues that are external may be variably dependant on the direct uptake of amino acids from the surrounding seawater (see Ferguson 1982 for review).

Amino acids have been shown to be effective chemosensory stimuli in fish, (Carpio 1978) crustaceans, (Carr 1978) and more recently to initiate appetitive behaviour in a number of echinoderms (described below). O. ophiura detected all ten amino acids and Betaine and in all cases the amino acids elicited behavioural responses in the animals in the form of the arm coiling response at certain concentrations. Collins (1974, 1975) indicated that L-Proline from corals was responsible for aboral arm coiling in the crown-of-thorns starfish Acanthaster planci and Moore and Huxley (1976), showed that a feeding response in the same animal could be elicited by single amino acids. Araki (1965) demonstrated that stomach eversion in Patira miniata was induced by individual amino acids and Zafiriou (1972)

showed single amino acids were weakly attractive to Asterias vulgaris. Valentincic (1975, 1978) working with Marthasterias glacialis demonstrated that the amino acids L-Cysteine hydrochloride and L-Proline were most stimulatory in feeding and L-Cysteine elicited searching behaviour or the appetitive phase of the feeding response. Only Castilla (1972) has reported that both individual and mixtures of amino acids have proved "repellent" to the behavioural animal Asterias rubens. It is difficult to understand why this should have occured in the light of all the other work where amino acids have appeared attractive to a number of other asteroids.

The neurones recorded from in this study are almost certainly a class of interneurones and not the sensory neurones themselves. The chemoreceptors in Ophiura ophiura are not located in specialized organs and are presumed to be distributed generally over the spines and tubefeet. The minimum threshold concentrations recorded in this study may therefore not be the absolute thresholds and it is possible that the thresholds for each sensory neurone are lower than those measured when recording from large interneurones. Excitation of the interneurones may well be a function of the overall input from the sensory receptors. It must also be remembered that the behaviour studies were performed on whole animals and not single arm preparations as in the electrophysiological experiments. The increased number of receptors available on the whole animal compared to a

single arm, may be critical in providing the needed excitation to produce an interneurone spike, and initiate behaviour in the whole animal. (The amino acids L-Proline and L-Serine were detected behaviourally at a lower concentration than when recording electrophysiologically, lending weight to the theory that even lower concentrations are being perceived at the receptor level). At present the transduction mechanism in echinoderm chemoreception is not understood.

The thresholds of detection for each amino acid and Betaine were consistent between preparations when recording electrophysiologically. The thresholds of behavioural response in the tank experiments however varied between individual animals and with the exception of L-Serine and L-Proline, were always at a much higher concentration. In general the arm coiling behaviour was most prevalent at the higher concentrations. Lower concentrations elicited only a movement towards the stimulus, suggesting that different behaviour in the animals is elicited at different concentrations. Reimer and Reimer (1975) working on four species of ophiuroids of the genus Ophioderma also showed that different concentrations of certain amino acids produced different behavioural responses. Again L-Proline elicited feeding behaviour, but the arm coiling response was considered by Reimer and Reimer to require both a chemical and mechanical stimulus. This was not the case in O.ophiura, where arm coiling was induced only by the presence of L-Proline in the water column and not by the addition of

mechanical stimulation of the tube-feet as well.

Amino acids present in the water column may signal a food source if detectable concentrations of these compounds are released by the natural foods of O.ophiura. In general carnivores and scavengers respond to commonly occurring amino acids emanating from prey tissue. The proteins of many marine invertebrates contain high levels of the amino acids L-Proline, L-Alanine, L-Valine, L-Leucine, and L-Serine. (Vegotsky and Fox 1962). In addition many marine invertebrates may excrete as much as 20-30% of total nitrogen indigested in the form of unchanged amino acids (Baldwin 1963). Polychaetes commonly encountered in the habitat of O.ophiura have been shown by Delauney (1913) and Fontes and Thouveny (1972) to concentrate free amino acids such as L-Proline, L-Alanine, L-Leucine and L-Serine extracellularly. The appetitive behaviour elicited by these amino acids in the brittlestars suggests that these chemicals may signal the presence of a familiar prey item in the environment.

Distance chemoreception provides information not only concerning prey recognition but also the identification of predators. The size of the predator and the threat it may present to these brittlestars may be signalled not only by certain compounds emanating from the individual predators, but also by the relative amounts released by the predator. Cessation of activity at the onset of predator recognition may prevent

detection. However, this behaviour is overridden by tactile stimulation from the predator resulting in the recommencement of motor activity and the rapid movement away of the brittlestar as desribed on page 40. This provides further evidence for a hierarchy of response present within the brittlestars dependant upon type and level of stimulus input.

The different phases in the sequence of response following detection of a food item have been variously described in other ophiuroid species. Hyman (1955) described the waving movements of the arms in carniverous species followed by movement towards the food. The arm coiling or arm loop has been described as a common method of purveying food to the mouth in Ophiura texturata (Von Uexkull 1905) Pectinura maculata and Ophiomyxa brevirama (Pentreath 1970) and species of Ophiocoma (Sides 1985). Gripping with the jaws while pushing away with the arms to tear pieces of the food item before ingestion was observed in Ophiura lutkeni (Austin 1966).

Distance chemoreception in O. ophiura was dependant upon directional chemical cues, if the food item was down-current from the animal then it was not detected. In non-cephalised animals such as brittlestars, where the chemoreceptors are presumably spread over much of the body, orientation towards the source of a chemical stimulus may be a result of the comparison of the level of sensory input between receptors located on various

parts of the body i.e. arm tips. Evidence for this is supported by the "zig-zag" pattern of movement of the brittlestar towards the food item. It is suggested that the animal moved in the general direction of the arm which received the greatest stimulation. i.e. that which was closest to the food item. If however, it wandered so that a different arm now received a greater input because it was closer to the source of stimulation, then it immediately orientated in this direction. Movement continued in this manner until the animal located the food item. Chemoreception of this type is dependant upon a continuous directional component. Reimer and Reimer (1975) demonstrated that if the chemical stimulus lacks a directional component uncoordinated behaviour in Ophioderma spp. may result.

CHAPTER 3

MECHANOSENSITIVITY

INTRODUCTION

Mechanical sensitivity is a basic property of all animals. It has been demonstrated in a number of marine invertebrates and these include Bryozoa, (Thorpe et al 1975) Ctenophora, (Horridge 1966) Chaetognatha, (Horridge and Boulton, 1967 Bone and Pulsford 1978) and Crustacea (Laverack 1963), for review see Laverack (1981). A great deal of interest has been centred on aquatic vertebrates such as fish, and the mechanical sensitivity of the acoustico-lateralis system, a unique, highly developed and diversified system of sense organs (for review see Hawkins and Horner 1981). Within the phylum Echinodermata, the sensitivity to mechanical movement has been inferred largely from behavioural experiments. Cobb (1968) described a sensory hillock on the globiferous pedicellaria of echinoids and Campbell and Laverack (1968) showed it to have a tactile function. However, more recently Campbell (1973) was unable to demonstrate a response to a water borne vibration stimulus in these pedicellariae. Certain echinoderms particularly crinoids and ophiuroids have been shown to respond to different current flow rates, (Meyer and Macurda, 1980 and Pentreath 1970).

In this chapter the mechanical sensitivity of O. ophiura is described using extracellular recordings from the radial nerve cord. The range that the brittlestar is able to detect in the mechanical mode

extends from the nearfield region of a vibrating source to the movement of the aquatic medium as a whole in the form of water currents. The behavioural responses of unfettered animals to stimulation in the same frequency range is described. The presence of an interoreceptor located within the spine, which is responsible for mechanical sensitivity is inferred from electrophysiological recordings in response to the movement of individual arm spines.

MATERIAL AND METHODS.

Electrophysiology.

Experiments were carried out on single arm preparations. Five oral plates and a portion of the adjacent lateral plates were removed to reveal the radial nerve cord (R.N.C.). The arm, except the distal 20 segments was immobilized, oral side uppermost in a perspex clamp. Recordings were made as described in previous chapters.

Vibration Stimuli.

The single arm preparation was placed in a two chambered dish containing filtered seawater, with the distal 20 segments isolated from the rest of the arm by the perspex wall dividing the dish. This ensured that only the distal portion of the arm would be stimulated and eliminated artefacts caused by displacement of the electrode due to water movement. The stimulus was produced by a vibrating perspex rod situated 5cm. away from the tip of the arm moving in an up and down direction. A Servomex waveform generator coupled to a 161 pulse generator produced sine waveforms at frequencies ranging from 1-50Hz. The animal was allowed to acclimatise for 1 hour in total darkness before commencement of the experiment. Ten minutes were allowed

between each stimulation to prevent possible habituation to the stimulus. Filtered seawater was used which was maintained at 6^{o} C. throughout each experiment.

Detection Of Hydrodynamic Disturbances.

The responses of the brittlestars to sudden hydrodynamic disturbances within the surrounding medium was investigated. Sudden movement of the water was produced by the downward movement of a perspex rod attached to a mechanical transducer. This was driven by a Servomex waveform generator coupled to a 161 pulse generator which produced single square waveforms. The water movement produced was in the form of a wave, 5cm. from the tip of the arm. Two particular aspects of movement in the surrounding medium were examined. Firstly the ability of the animal to detect water movement of different magnitudes and secondly the ability to detect different rates of movement of the surrounding water. Difficulties were encountered when quantifying the magnitude of water movement due to the complexities involved in measuring waveforms in an enclosed space. The relative intensity of the wave was expressed in terms of the amount of water displaced by the rod in a given time. Similarly the rate at which the wave moved was measured in terms of the rate at which the probe moved through the water over a given distance. The experiments were designed to test the animals ability to perceive relative change in the surrounding environment and not to

quantify exact thresholds. It was felt that the limitations of the experimental system precluded reproducing stimuli that would be significant in an unfettered animal in a natural environment. Increasing magnitudes of waves were produced by increasing the amount of water displaced from the values of $19mm.^3$ - $98mm.^3$ in a 0.5s.period. The rate of movement of the wave was assigned values of between $3mm.sec.^{-1}$ - $30mm.sec^{-1}$. Similar experiments were carried out on single arm preparations where all the arm spines on the distal 20 segments of the arm had been removed. This was achieved using fine watchmakers forceps to break the spines at the base. Two hours were allowed for the animal to recover before experimentation. 25 preparations were experimented on and each produced consistent responses to the stimuli for up to 8 hours after the removal of the spines.

Movements Of Individual Spines.

Recordings were made from the radial nerve cord in response to movement of individual lateral arm spines. Experiments were performed on sections of an isolated arm preparation which was immobilized using perspex clamps either side of the lateral spine examined. A wax cap (1mm. in diameter) attached to a fine dissecting pin was placed over the tip of a lateral spine. An electro-mechanical transducer attached to the pin was used to move the spine in the required way. When the

spine was lying flat against the surface of the arm it was assigned an arbitary value of 0^{o}. The tip of the spine was moved outwards from the arm at a constant rate to angles from 1-30^{o}, measured relative to its initial position. The spine was also moved from its extended position through angles from 30^{o} - 1^{o} back to lie flat against the arm. The rate at which the spine was moved through a given angle (20^{o}) was varied between 1 second and 0.1 second. All the spines except the stimulated one were removed, and experiments were carried out in total darkness using filtered seawater maintained at a constant 6^{o}C. Ten minutes were allowed between each experimental movement. Each preparation was allowed to acclimatise in the dark for 1 hour.

Detection Of Different Flow Rates.

The brittlestars ability to discriminate between different rates of water flow was examined. Filtered seawater at a constant 6^{o}C. was passed continuously from a constant head reservoir over the distal 20 segments of a single arm preparation at a rate of 3.0cm.sec^{-1}. The preparation was allowed to acclimatise under these conditions in total darkness for 1 hour. The addition of water from the flow regulator (Fig.1) increased the rate of flow passing over the arm tip. The amount of water from the flow regulator was varied by aligning one of six different diameter holes drilled through the floor of the regulator with the outflow. The rate of flow was

FIG. 1.

Diagram of the apparatus used to vary the water flow passing across the tip of a single arm preparation. Filtered seawater flows from a constant head reservoir (A) regulated by a small tap (R) and bypasses the intermediate reservoir (C). The intermediate reservoir contains six holes of different diameters bored through the floor of the cylinder. Water flow is increased by revolving the top portion of the reservoir to connect one of the holes with the outlet (O) adding water to the flow leading to the arm tip. (S).

Not to scale.

A
R
S
O
C

increased in steps up 3.6cm.sec^{-1}. After each increase in flow the animal was allowed to acclimatise for 20 minutes with the flow rate at 3.0cm.sec^{-1}.

Detection Of A Predator.

The examination of plaice (Pleuronectes platessa) gut contents, (Moore 1982) showed that O.ophiura composed 19% by wet weight of the total gut contents and may be considered to be a significant food component in this flatfish. Experiments were carried out to investigate whether the brittlestar was able to detect the movement of a plaice by the hydrodynamic disturbance it caused. Two sizes of plaice were used, 3cm. and 9cm. in length measured from the snout to the fork of the tail. Fish of this size were chosen because of their availability and the limitations in size of the experimental apparatus. Recordings were made as each plaice swam along a perspex barrier placed at right angles to the arm tip and adjusted so that the nearest part of each plaice was always 5cm. from the brittlestar. Each plaice was positioned at one end of a perspex barrier running at right angles to the tip of the arm. The barrier could be adjusted so that the nearest part of each plaice was always the same distance from the arm between experiments. Recordings were made as the plaice swam along the barrier past the brittlestar arm. The experiments were replicated until recordings from 20 different animals were obtained for each sized plaice

swimming at the same rate measured by a stopwatch.

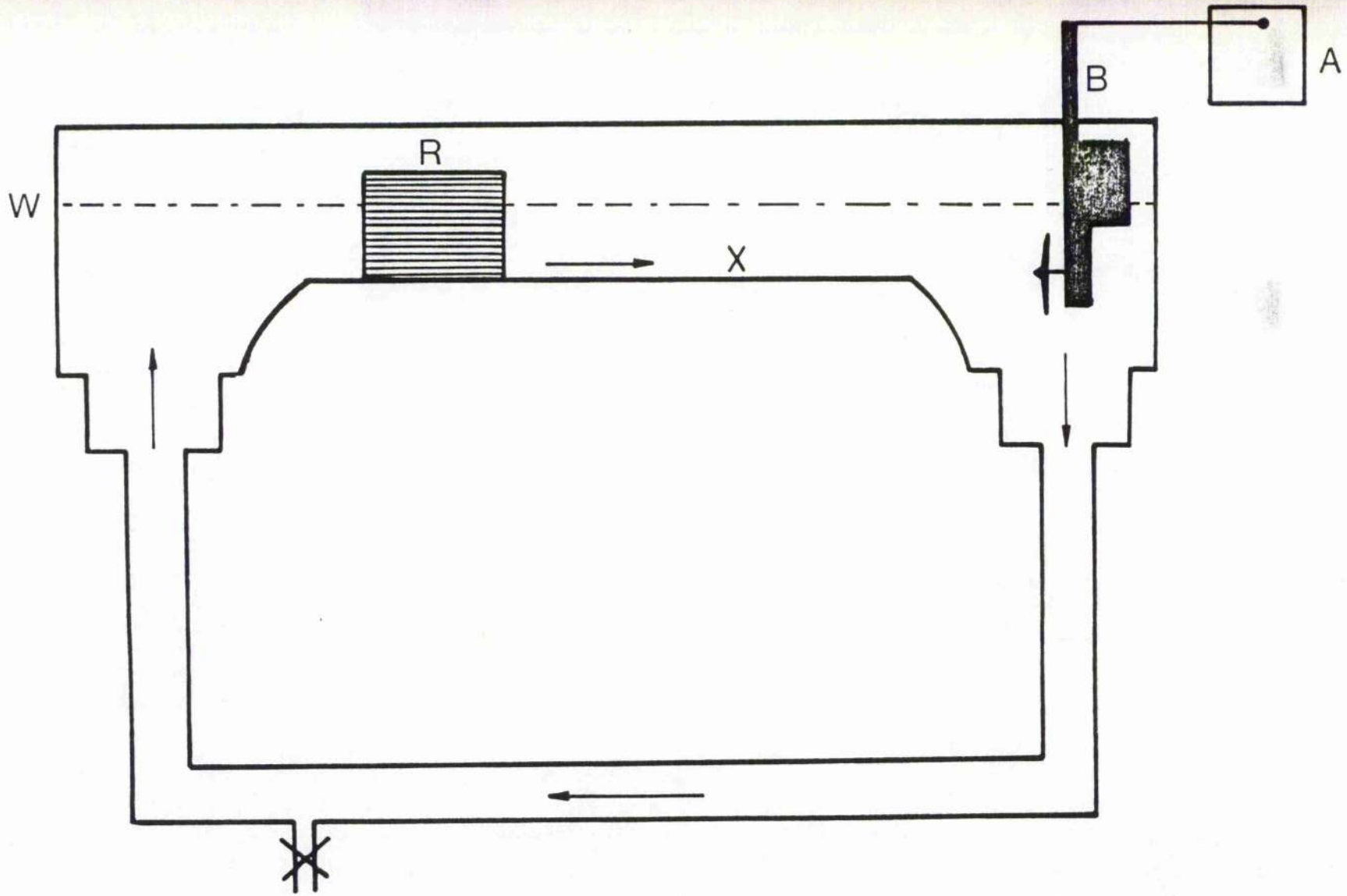
Behaviour.

The behaviour of unfettered animals in laboratory tanks in response to the stimuli presented in the neurophysiological studies was observed. Single specimens were placed in a small tank of the same dimensions used in the neurophysiological studies and allowed to acclimatise for 1 hour, illuminated by a single red photographic lamp. The behaviour of both active and inactive specimens were recorded in response to the perspex rod vibrating at frequencies of between 1-50Hz. The responses of the brittlestars to pressure waves of increasing magnitudes and rates of movement passing across the animal were also recorded. Twenty minutes were allowed between each stimulus to prevent habituation of the response. Experiments on the discrimination between different water flows were carried out on animals in a large flume (Fig.2). The behaviour of 60 animals to increases in the water flow which corresponded to those studied in the neurophysiological experiments, were observed under the illumination of a red photographic lamp. Animals were placed in the flume and allowed to acclimatise to a flow rate of 3.0cm.sec^{-1}, for 15 minutes or until each remained quiescent on the floor of the flume for 5 minutes, in the characteristic arm posture, with the distal fifth of each arm extended upwards. The flow was increased and then

FIG. 2.

Diagram of the apparatus used to record the behaviour of brittlestars in response to water flow. The speed of the propellar attached to the outboard motor (B) is increased via the variable speed motor (A). Filtered seawater is circulated in the direction of the arrows and turbulence reduced by densely packed plastic straws (R) before passing across the animal (X). The water depth is 25cms. (W). Side view.

Not to scale.


A
B
R
W
X

the behaviour of each animal noted. Five minutes was allowed between each flow increase during which time the rate was returned to a constant 3.0cm.sec^{-1}.

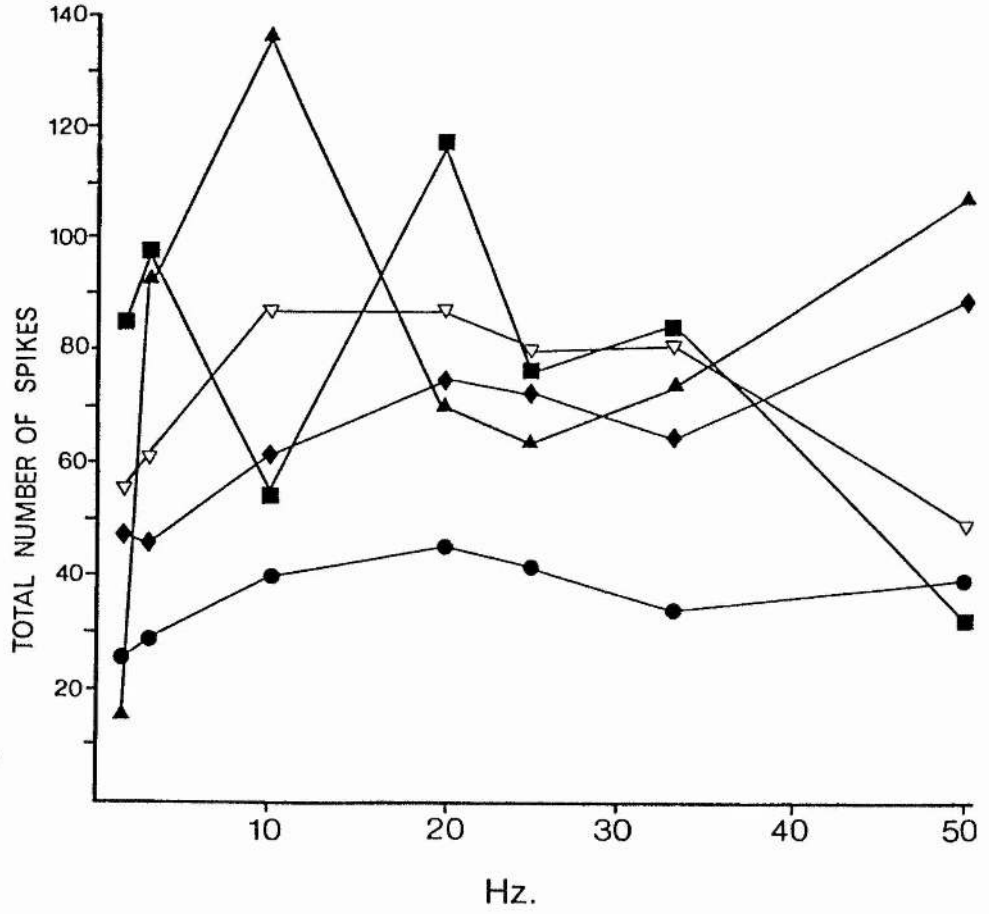
RESULTS.

Vibration Stimulus.

The responses of five different preparations to a range of frequencies of between 1-50Hz. are illustrated in (Fig.3). Typical extracellular recordings at different frequencies show two distinct patterns of response (Fig.3a & b). Frequencies of vibration of 1-20Hz. inclusive produced typical patterns of response (Fig.3a). The response is characterized by the presence of numerous small amplitude spikes (10-15μv), few spikes above 35μv and the absence of spike bursts. This type of response is similar to recordings made during the lateral flexure of the unrestrained arm tip. At frequencies above 20Hz. the pattern of recorded response consisted of a complex initial burst of single unit activity and thereafter discrete bursts of spikes the largest between 40-45μv (Fig.3b). The pattern of response was similar to those recorded to a shadow stimulus.

FIG. 3.

Graph showing the typical responses in terms of total number of recorded spikes of five individual animals out of 25 preparations to a range of frequencies of vibration. (1-50Hz). a) Extracellular recording from the R.N.C. in response to frequencies of vibration of between 1 and 20Hz. b) Extracellular recording from the R.N.C. in response to frequencies of vibration of above 20Hz. Note the two different patterns of response. The lower arrow indicates the initiation of the stimulus.


140
120
100
80
60
40
20
TOTAL NUMBER OF SPIKES
10
20
30
40
50
Hz.


a
b
1s

Hydrodynamic Disturbance Stimuli.

The movement of the perspex rod and subsequent wave which passed across the tip of the arm resulted in a burst of spikes recorded extracellulaly from the radial nerve cord. Each response consisted of a variable number of units between 10-45μv. dependant upon stimulus. Increasing the magnitude of the pressure wave elicited a corresponding increase in the size and number of unit potentials within the recorded response (Fig.4). The initial burst of spikes in the pattern became more complex with increasing magnitude and regular spike bursts were evident. The greater magnitude waves resulted in a pattern of response that is similar to the shadow response. Increasing the rate at which the pressure wave moved across the arm tip resulted in an increase in the size of recordable spike, but a decrease in the number of smaller spikes within the response (Fig.5). The largest spike measured around 40μv. No response was elicited from the R.N.C. to movement of the waves of any magnitude once the spines had been removed (Fig.6). The shadow response could still be recorded however indicating that the suction electrode remained attached to the R.N.C. subsequent to the removal of the spines and the nervous system remained functioning. This also indicates that the shadow response is not wholly associated with the spines.

FIG. 4.

Extracellular recordings from the R.N.C. in response to single waves of increasing magnitude passing across the tip of a single arm preparation. The displacement of a) $19cm^3$. b) $39cm^3$. c) $78cm^3$. d) $98cm^3$. of water over a 0.5 second period produces the waves of increasing magnitude. The lower trace indicates the moment of application of the stimulus.

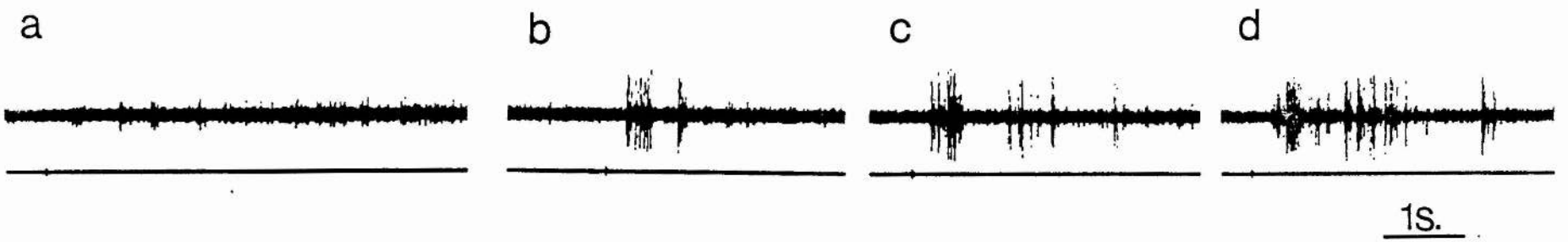

a
b
c
d
1s.

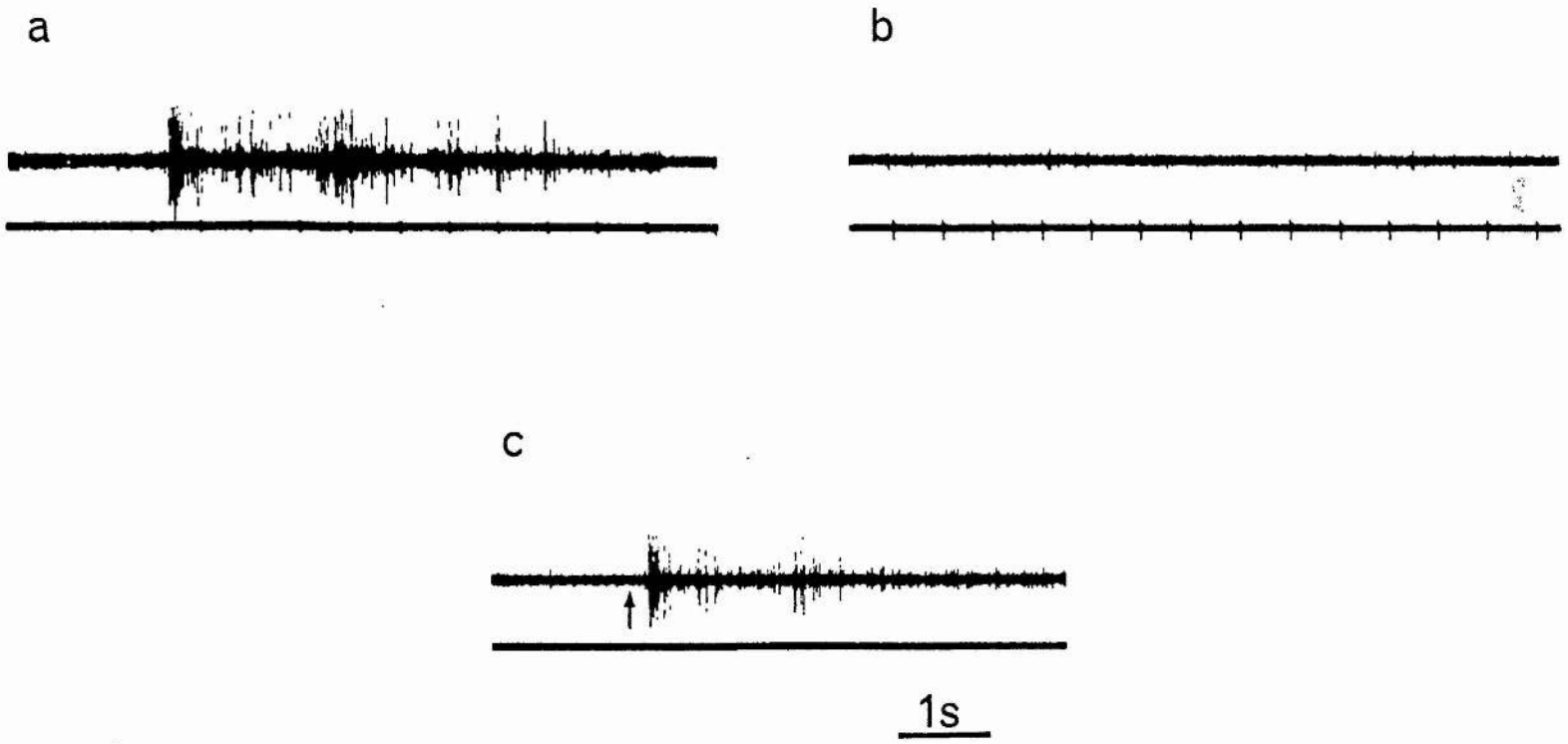
FIG. 5.

Extracellular recordings in response to single waves passing across the arm tip at increasing rates. a) 3mm.sec^{-1}. b) 12mm.sec^{-1}. c) 20mm.sec^{-1}. d) 30mm.sec^{-1}. The lower trace indicates the moment of application of the stimulus.


a
b
c
d
1s.

FIG. 6.

a) Responses recorded extracellularly in response to the repeated displacement of 78mm of water in a 0.5 second period to produce single waves resulted in a complex train of single unit activity from an intact single arm preparation. b) Same stimuli presented to the single arm preparation after the arm spines have been removed, resulted in no recordable activity from the R.N.C. c) The shadow response recorded from the preparation with the spines removed indicate that ablation of the arm spines had not affected the ability to record responses from the R.N.C. The lower trace in a) and b) and the arrow in c) indicate the initiation of the stimuli.


a
b
c
1s

Movement Of Individual Spines.

The movement of a single lateral spine produced a response in the form of a number of single unit potentials that were recordable from the R.N.C. of the brittlestar. Both the number and the amplitude of recorded spikes increased as the angle through which the spine was moved increased (Fig.7). The movement of a spine 10^{o} relative to its initial position resulted in a single potential being recorded (amplitude 15µv) whilst movement through an angle of 25^{o} produced a complex burst, recordable from the nervous system with the largest unit approximately 45µv. Increasing the rate at which a single spine was moved through a given angle elicited a corresponding increase in the recordable response in terms of both number and amplitude of unit (Fig.8a,b,c). In all the recordings there was an obvious lag period between the onset of the stimulus and the recorded response. The duration of this lag period was dependant upon the rate at which the spine was moved. The faster the spine was moved, the larger the recorded potential and the greater the decrease in the time between stimulus and recorded response. This suggests that the larger diameter neurones from which the spikes are recorded may have a greater conduction rate than the smaller neurones. Further experiments were carried out where two individual lateral spines, on adjacent segments were moved simultaneously. Increasing the rate at which the two spines were moved through a given angle

FIG. 7.

Extracellular recordings from the R.N.C. in response to the movement of individual lateral spines, through angles of a) 10^0 . b) 15^0 . c) 20^0 . d) 25^0 . relative to their initial position lying flat against the arm of the animal. The lower trace indicates the moment at which the spines were moved.

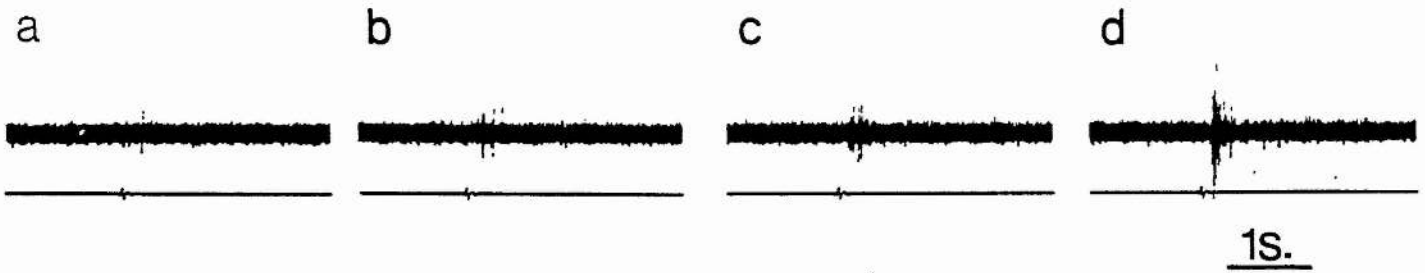
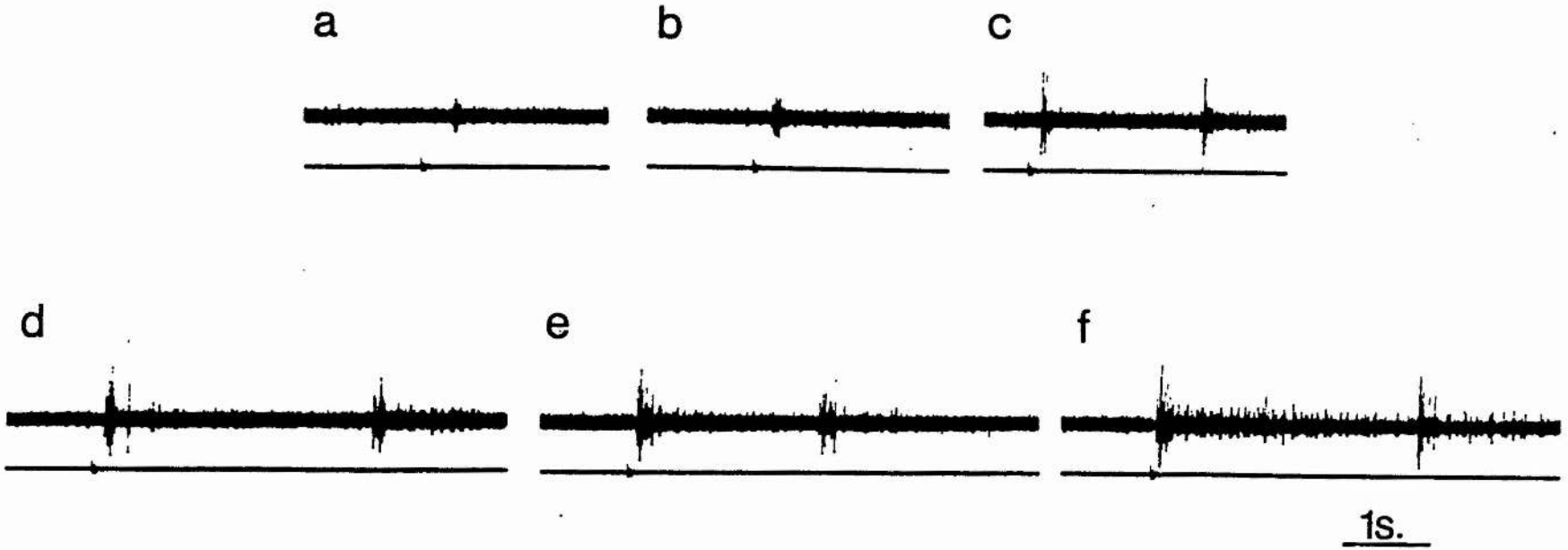

a
b
c
d
1s.

FIG. 8

Responses recorded to the movement of individual spines at increasing rates of a) 1 second. b) 0.6 second. c) 0.1 seconds through an angle of 20^{0} measured relative to their initial position. d), e) and f) are the recorded responses to the simultaneous movements of two adjacent spines at the same rates. The second response in each record is the movement of the spines back to their original positions. The lower traces indicate the points at which the spines were moved.


a
b
c
d
e
f
1s.

(Fig.8d,e,f) elicited not only a corresponding increase in response with rate, but an increase in the response when compared to movement of a single spine at corresponding rates. A similar burst of spikes was recorded when the spines were moved back to their original positions lying flat against the surface of the arm.

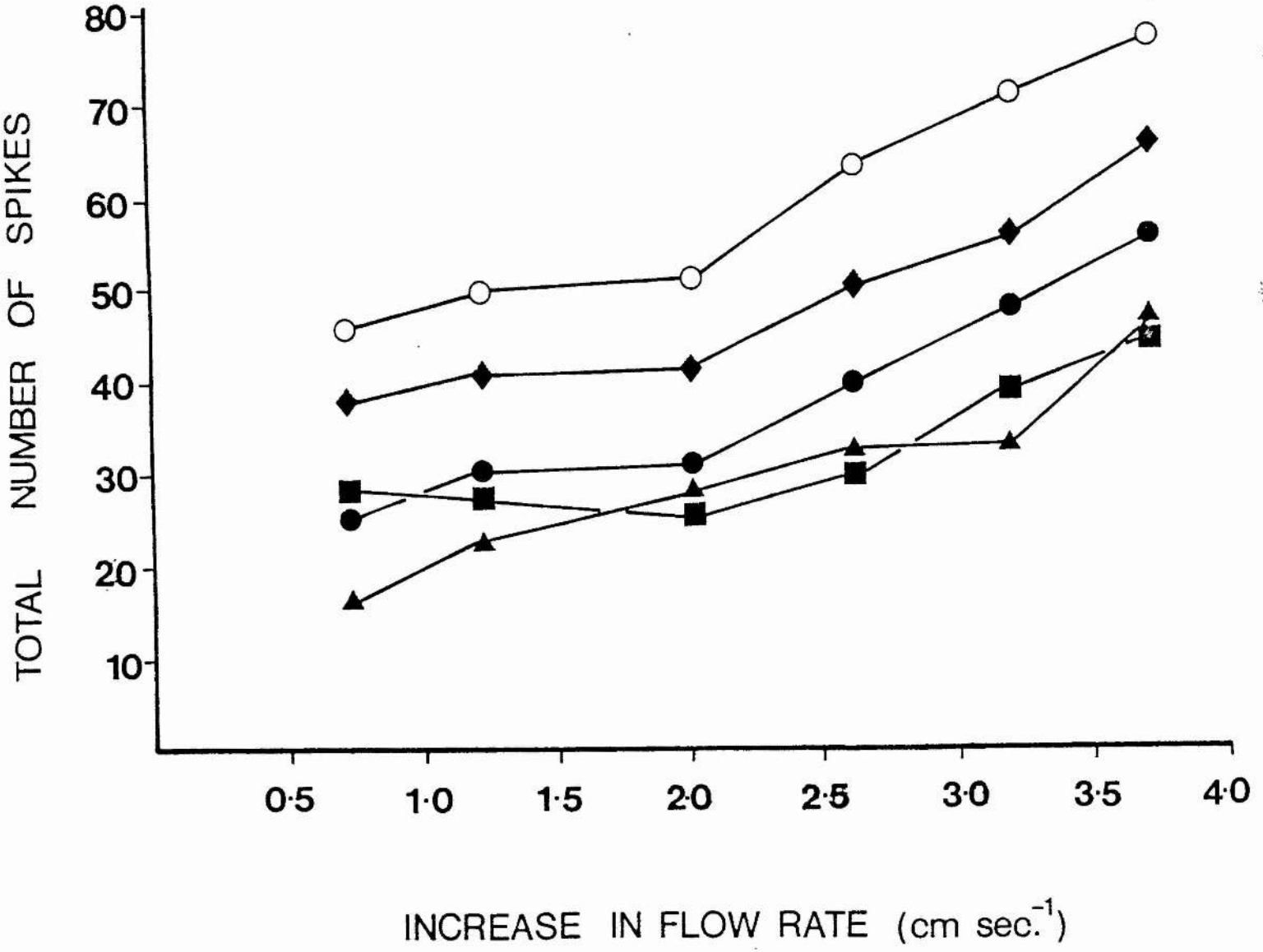
The same experiments were carried out on single arm preparations where all surface structures and epithelium from the spines and arm were stripped away using a 50% solution of domestic bleach. Later examination under the S.E.M. and T.E.M. verified this. Under these circumstances responses were still recorded from R.N.C. to movement of single lateral spines, both through increasing angles and at increasing rates.

Detection Of Flow Rate.

Increasing the flow rate elicited a corresponding increase in the response recorded from the R.N.C. of the brittlestars (Fig.9). The response was in the form of a burst of single unit potentials (30μv) recorded at the onset of the flow rate increase, the number of which increased with rate. An increase of only 0.7cm.sec.$^{-1}$ was sufficient to produce detectable activity from the R.N.C.

FIG. 9.

Graph illustrating the typical responses of five individual animals out of 25 tested to increases in water flow. The total number of spikes present in each extracellular recordings is plotted against increases in the flow rate of the water.


TOTAL NUMBER OF SPIKES
80
70
60
50
40
30
20
10
0·5
1·0
1·5
2·0
2·5
3·0
3·5
4·0
INCREASE IN FLOW RATE (cm sec.$^{-1}$)

Detection Of A Predator.

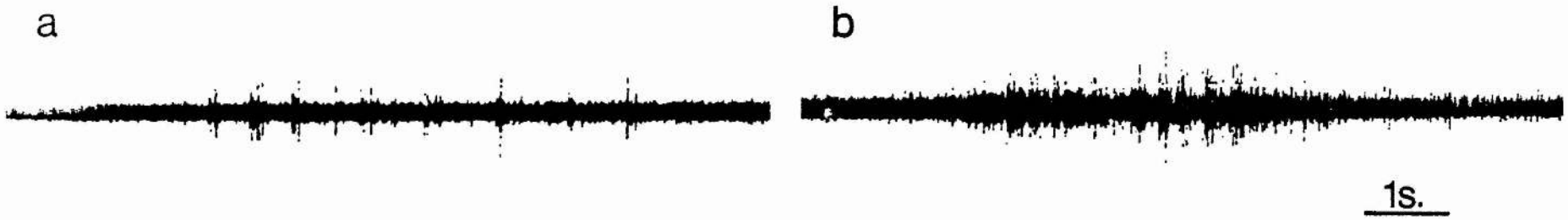
Single unit activity in response to the movement of the different sized P.platessa was recordable from the R.N.C. (Fig.10). The responses differed in terms of size and number of recorded spikes. The movement of the larger 9cm. P.platessa elicited the greater response with spike amplitudes of approximately 30μv. However it is not clear whether the responses are due entirely to hydrodynamic disturbance caused by the movement of the plaice or if there is a chemosensory component present. O.ophiura has been shown to be able to detect P.platessa by distance chemoreception. These experiments must be regarded as preliminary and further experiments are necessary.

Multiple Electrode Recordings.

Simultaneous recordings from two different positions on the R.N.C. in response to a perspex rod vibrating at a frequency of 25Hz. resulted in responses that were similar in pattern at the two recording sites. (Fig.11). This indicates that the response to this mechanical stimulation is carried to all parts of the animal in a substantially unaltered form. Spikes ranged in amplitude from 10-35μv.

FIG. 10.

Typical extracellular recordings from the R.N.C. in response to the movements of two different sized Pleuronectes platessa. a) 3cm. b) 9cm. The length of fish measured from snout to fork of the tail. Note the greater response in terms of size and number of recorded spikes to the larger sized fish.


a
b
1s.

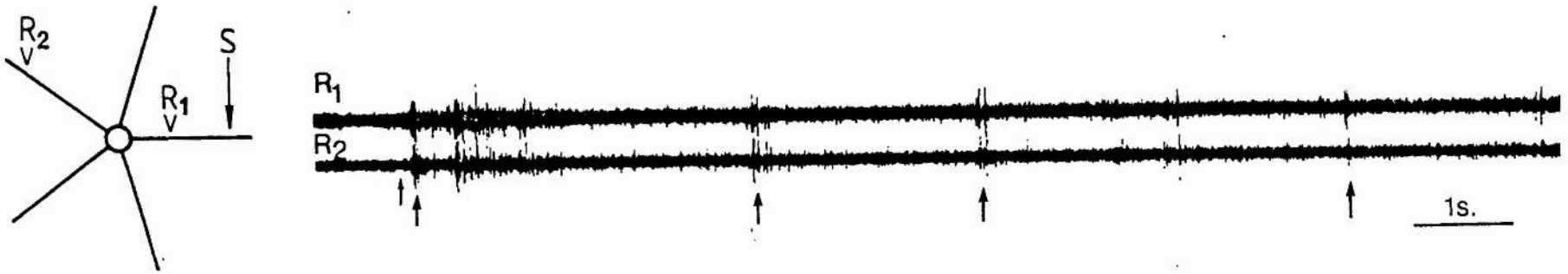

R2
V
S
R1
V
R1
R2
1s.

FIG. 11.

Simultaneous extracellular recordings in response to a perspex rod vibrating at 25Hz. frequency for 20 seconds. S denotes the arm tip stimulated and R_1 and R_2 are the electrodes positioned on different arms. The stimulated arm is physically isolated from the rest of the animal by a perspex barrier to prevent detachment of the electrodes by the water movement. The arrows indicate groups of units that are similar at both recording sites. The response to mechanical stimulation is conducted to the other arms in a substantially unaltered form.

Behaviour.

The brittlestars exhibited two distinct behavioural responses at different frequencies of vibration produced in the surrounding water. Firstly, the perspex rod vibrated at frequencies of 20Hz. and below proved attractive to the animals. Quiescent animals moved rapidly towards the source of stimulus, waving the distal portions of the leading arms. The rod vibrated at frequencies of above 20Hz. had no effect on the behaviour of quiescent individuals, but animals exhibiting any type of movement immediately ceased all activity at the onset of the stimulus. This freezing of the animal was the same response which was elicited by the detection of a shadow stimulus. The significance of this observation to O.ophiura is discussed on page 66.

The same two behavioural responses were exhibited by the brittlestars at different magnitudes and rates of movement of pressure waves produced close to the tip of the animal. In general water movements of low magnitude and slow rates, (arbitary values of $19mm^3$ and 6mm.sec-1 and below), elicited movement in the animals towards the source of stimulus. Higher magnitude, faster moving pressure waves, (arbitary values of above $19mm^3$ and 6mm.sec-1), produced no response in the behaviour of quiescent animals but brought about the immediate cessation of activity in any specimen showing any type of movement. The "freezing" of the animal occurred at the

TABLE 1: Behaviour of O.ophiura in response to increases in the rate of flow of water.

Increase in flow rate ($cm.sec^{-1}$).	No Response %	Arm Waving %	Movement Downstream %	Movement Upstream %
0.7	85.0	1.6	6.7	6.7
1.2	68.3	6.7	10.0	15.0
2.0	60.0	5.0	10.0	25.0
2.6	33.3	8.4	8.3	50.0
3.6	20.0	6.7	3.3	67.0

Flow rate at a constant $3.0cm.sec^{-1}$ before each increase in rate.
Numbers of animals responding as a percentage of the total number of animals.

N=60

onset of the stimulus.

The experimental animals performed three distinct types of behaviour to an increase in the flow rate (Table 1.) namely, attraction towards or away from the direction of water flow and the waving of the arm tips in the water column. Animals placed in the flume before experimentation at a flow rate of 3.0cm.sec.$^{-1}$ showed no movement towards or away from the direction of flow. All animals remained quiescent in the characteristic arm posture, with the arms flexed aborally to extend up into the water column. The total number of animals showing changes in behaviour increased as the flow rate was increased. Positive rheotactic behaviour was the most common response to increasing water flow. Whereas all the preparations in the neurophysiological studies showed a recordable response at a flow rate increase of 0.7cm.sec.$^{-1}$, only a small percentage indicated that they had detected this increase by behavioural changes.

No behavioural response was elicited from unfettered brittlestars to the movement of the two plaice within the experimental tank. Animals which were quiescent remained so and brittlestars showing any type of movement continued to do so. This is discussed on page 66.

DISCUSSION

The sensitivity of O.ophiura to movement in the aquatic medium extends from detection in the near field region of a vibrating source through the far field propagated pressure wave to the movement of the environment per se in the form of water currents.

The frequencies of vibration detected by the brittlestar fall into a range which is similar to those detected by other marine invertebrates and lies at the very lowest periodicity 1-100Hz. Horridge (1966) demonstrated the sensitivity of the fingers of Leucothoe to vibration; Josephson (1961) reported that the hydranth of the hydrozoan polyp Syncoryne bent towards a small vibrating object placed close by and Thorpe (1975) demonstrated the sensitivity of colonial bryozoan to low frequency movements. However these authors provided no explanations for this behaviour. Horridge and Boulton (1967) demonstrated that chaetognaths were attracted to a vibrating source between 9-20Hz. but above these frequencies the animals performed an escape response. Campbell and Laverack (1968) investigated the response of various pedicellariae on E.esculentus to vibrations generated by an oscillator but found that the frequencies used were all ineffective in evoking closure of pedicellariae. It is difficult to correlate the vibration stimulus presented under laboratory conditions

with any known stimulus occurring in the brittlestars natural environment. Josephson (1961) and Horridge and Boulton (1968) suggest that the lower frequencies may relate to movement of prey such as planktonic crustacean. Schoener, (1971) Dearborn, (1977) Macurda, (1976) and Tsurnamel and Marder (1966) discuss the possibility that carnivorous ophiuroids capture planktonic species and small swimming organisms in an arm loop. This would require the initial detection of the prey item possibly by the detection of the hydrodynamic disturbance caused by the animals movements. O.ophiura is unable to feed in this way because of the stucture of the arms and would therefore have little advantage in detecting prey in such a manner. The significance of detecting water borne vibrations is therefore not clear.

The cessation of activity in O.ophiura has been related to predator avoidance and the same behaviour in response to the higher frequencies of vibration may be an indication that the brittlestar relates this type of stimulus to some form of threat, i.e. movement of a flatfish. Similarily, sudden intense movements of the surrounding water in the form of fast moving propagated pressure waves indicate a localised water disturbance. If caused by a predator immediate cessation of activity may increase the animals chances of survival by escaping detection. The ophiuroid has been shown to detect movement of flatfish neurophysiologically, but whether the fish used were of a size to present a threat and elicit a response is doubtful. This is supported by the

analysis of P.platessa gut contents, (Moore 1982). O.ophiura was found only in the stomachs of plaice 249mm. or more in length, suggesting that only large plaice were predating on the brittlestars. Cessation of activity of the arm tips when the rest of the animal is buried in the sand, may also increase the chances of the animal escaping partial predation of these parts. Over 75% of the animals collected in the trawl showed signs of regenerating arms suggesting that partial predation occurs in natural conditions. Horridge and Boulton (1968) gave no expla nation for the escape response in chaetognaths at the higher frequencies. Recent work on copepod plankters by Szlauer, (1965) Drenner et al,(1978) Haury et al (1980) has indicated that several species recognise predators and prey by the pattern of the hydrodynamic disturbance produced. In an animal such as O.ophiura which is able to discriminate quite finely between different types of water movement it is likely that similar capabilities may exist. It would be interesting to record from the R.N.C. the responses if any to the presence of copepods and other planktonic species in the water close to the brittlestars.

The behaviour of the different classes of echinoderms in response to water flow and currents has received varying amounts of attention in the literature. Much of the work has described the relationship between flow and feeding mechanisms and has been based on animals such as the crinoids which are dependant upon environmentally produced water movements to carry

suspended food particles to the feeding organs (see Meyer 1982 for review). The ophiuroids have also received some attention (for review see Warner 1982), but the holothurians, (Massin 1982) echinoids, (De Ridder and Lawrence 1982) and the asteroids (Jangoux 1982) have attracted little interest, mainly because of their reliance on mechanisms other than suspension feeding. The position O. ophiura maintains in currents of low flow rate, with the arms flexed upwards into the water column, is similar to the feeding posture of the mucous spine feeding species Amphipholis squamata and Ophiopteris antipodum (Pentreath 1970) and the mucous net feeding species Ophiocomina nigra (Fontaine 1965). Ophiuroid species that filter feed with the aid of their tube feet also orientate their arms upwards into the current (Masse 1963, Buchanan 1964, Fricke 1970, Pentreath 1970, Warner and Woodley 1975, Olscher and Fedra 1977). Whether O. ophiura is feeding in this way, or is extending the parts where the chemoreceptors are located into the current to act in the role of distant chemoreception is not known. The positive rheotactic behaviour of the animals to increases in rate of water flow suggests the latter. Although filtered seawater was used, the presence of free amino acids in the water and the increase in the rate at which they reach the receptor sites with flow may be sufficient to release searching behaviour by the animals in the direction of the source of stimulus. Castilla (1971), Castilla and Crisp (1970) and Castilla and Crisp (1973) have shown positive rheotactic behaviour in Asterias rubens but the authors

came to no definite conclusions as to the reason for this behaviour. O.ophiura was able to detect an increase in the flow rate of 0.7cm.sec^{-1}. Castilla and Crisp (1973) showed that A.rubens detected as little as a 0.15cm.sec.$^{-1}$ increase in water movement. Although neurophysiologically the brittlestars detected this level of 0.7cm.sec.$^{-1}$ consistently, very few animals showed any behavioural change to this stimulus. Above this level animals showed a positive rheotactic response to increase in the water flow. Increases in the rate of water flow have also been shown to release different behavioural responses in a number of filter feeding ophiuroids. Feeding behaviour is elicited in Ophionereis fasciala by current speeds of 5-10cm.sec.$^{-1}$ but the arms are withdrawn at 10-15cm.sec.$^{-1}$ (Pentreath 1970). Similarly Ophiothrix fragi lis feeds in currents up to 25cm.sec.$^{-1}$ but above this speed individuals progressively "crouch down and link arms" to avoid being swept away, (Warner and Woodley 1975).

Neurophysiological experiments apparently indicate that detection of movement within the aquatic environment is facilitated through the movement of the spines of the arm as a whole and not by the deflection of any cuticular processes such as cilia. This suggests the presence of an interoreceptor which has not been described in the Echinodermata before. Anatomical evidence for the existence of such a receptor is lacking because of the difficulty in locating what may be only a single nerve process located in the base of the spine. Castilla and

Crisp (1973) imply that specific water current receptors may be present in Asterias rubens but as yet these sense organs have not been reported.

CHAPTER 4

ANATOMICAL STUDY OF PRESUMPTIVE SENSORY STRUCTURES

INTRODUCTION

Complex receptor structures are scarce within the echinoderms. Apart from the optic cushion, a pigmented sense organ on the oral surface of some asteroids,(see Penn and Alexander 1980), it is considered that sensory reception occurs over much of the body surface of echinoderms. Millott (1975) for example, proposes a general dermal light sense in echinoids. Structures with modified cilia present have been described in all classes of echinoderms and recently Whitfield and Emson (1983) described a structure on the spines of Amphipholis squamata very similar to a "stabchen", first described by Reichensperger (1908) and proposed it had a chemoreceptive function. There are other studies on receptors in the pedicellariae of echinoids concerned with the sensory hillock (Cobb 1968, Campbell 1973 and Oldfield 1975).

This chapter describes four types of presumptive receptors located on the spines, tubefeet and general body surface of O. ophiura. These structures are related to the perceptive abilities of the brittlestar described in previous chapters. The discussion compares the structures of these receptors with those described previously in the phylum and correlates these findings with what is known neurophysiologically and behaviourally.

MATERIALS AND METHODS

Transmission Electron Microscopy.

Tissue was fixed by immersion in a 5% solution of glutaraldehyde in 0.1M. Sorensen's phosphate buffer (pH 7.4). Ten seconds later an equal volume of a 2% solution of osmium tetroxide in 0.1M. phosphate buffer was added and mixed. The tissue was left for 15 minutes and then washed in distilled water. Tissue containing calcite was then decalcified for 24 hours in a mixture of 2% solution of ascorbic acid and 0.3M. NaCl. (see Dietrich and Fontaine 1975). All tissue was then rapidly dehydrated in acetone and embedded in Araldite. Sections were stained with lead citrate and uranyl acetate and examined using a Phillips 301 transmission electron microscope.

Scanning Electron Microscopy.

The tissue was fixed by immersion in a 5% solution of glutaraldehyde in fresh seawater and ten seconds later an equal volume of a 2% solution of osmium tetroxide in fresh seawater was added and mixed for 20 minutes. Tissue was then washed in fresh seawater (4^{o}C.) for 1 hour. The tissue was then dehydrated using acetone, critically point dried and sputter coated with gold. The

tissue was viewed using a Jeol JSM - 35CF Scanning Electron Microscope.

RESULTS

Tubefoot Of Ophiuroids.

Scanning electron microscopy revealed the surface of the tubefeet of Ophiura ophiura to be covered with microvilli caps and other larger more complex structures (Fig.1). Examination in detail (Fig.2) revealed three particular structures. Firstly there were pores between 1µm and 2µm in diameter that almost always contained an accumulation of material. There were also raised areas about 1µm high of approximately the same size that contained a small pore or occasionally a short projection which was of a size compatible with it being a cilium. Finally there were small non-projecting structures of less than 0.4µm diameter which were only obvious upon close examination. The larger pores were usually in pairs and covered much of the tubefoot surface, the small non projecting structures were always associated with these pores and usually lay adjacent to them. The larger projections were present over the whole tubefoot surface but had a more irregular and much sparcer distribution.

Transmission electron microscopy revealed the pores to be the openings at the surface of secretory cells and the 2 other structures to be putative sensory receptors. Figs.3 and 4.

Secretory Cells.

The tubefeet contained large numbers of secretory cells which were flask shaped with a narrow neck projecting at the surface. A number of microtubules passed longitudinally and were present round the circumference of the narrow neck. These are illustrated in Figs.5 and 6. The cells had a loose packing at the surface but at a depth of 3 to 4 microns below the surface they were bound together by intermediate and septate junctions.

'Stabchen' Receptors On Tubefeet.

The most obvious putative receptor cell had a similar structure to the 'stabchen' described by Whitfield and Emson (1983) on the spines of the brittlestar Amphipholis squamata. This is illustrated in longitudinal sections in Fig.4. A narrow cell projected towards the surface and terminated in a cilium surrounded by a ring of specialized microvilli. Two sets of microvilli were present similar to those descibed by Whitfield and Emson (1983) and these are illustrated in transverse section on the spines in Fig.16. The cilium was characterized by a root up to 4 or 5µm long and there was invariably a secondary basal body or centriole at right angles to this. Satellite material from the basal body of the cilia appeared to be attached to the base of

the microvilli that surrounded the cilia. The cilia in longitudinal sections do not project beyond the ring of specialised microvilli at the surface and the cytoplasmic detail was often amorphous and difficult to categorize. The ring of microvilli projected about 1µm from the tubefoot surface cuticle to form a collar.

Non Projecting Receptors.

These receptors were not readily apparent using the scanning electron microscope nor was their description easy from longitudinal transmission sections. Fig.3 shows in longitudinal section what may have been one of these structures but it is quite possible that this was a glancing section through the `stabchen' type of receptor. Sections that were serial and horizontal to the tubefoot surface however revealed the fundamental differences from the `stabchen' receptor and showed a second type of receptor that was not surrounded by a collar of specialized microvilli and did not project above the cuticular surface.

Figs.8, 10, 11, 12 and 13 are horizontal sections across the surface of tubefeet and spines. The fixation of tubefeet that were not decalcified was much superior in the wealth of cellular detail Fig.8. It was immediately clear that there were many more transverse sections of ciliary structures present than could be accounted for by the sparse distribution of `stabchen' on

the tubefeet. It was obvious that there were generally a pair of cilia for each pair of mucous gland openings. The structure of each cilia was however highly variable, some, Fig.7, were 9+2 but most were not. Serial section showed that the 9+2 configuration occurred immediately above the basal body but was usually rapidly lost to a 9+0 configuration (Fig.8). Many of the cilia were not surrounded by the specialized microvilli of the `stabchen' type (for description see Whitfield and Emson 1983) and when followed to the surface ended level with the cuticle. The microtubular array at this level was always irregular (Fig.10). A re-examination of material in longitudinal section (Fig.9) indicated that these presumed receptors did not protrude through the cuticle. Such structures are not artefacts of plane of cut when examined using serial sections. A close examination of material prepared for scanning microscopy revealed many of them associated with the pairs of sensory pores (Fig.2). They were numerically much more common than `stabchen' type receptors outnumbering them by a factor of about 10:1. All types of receptor had a secondary basal body associated with the cilium. This contained the typical triplet arrangement of microtubules. The non projecting receptors also showed irregular microtubular arrays within the cilium. Near the basal body this was normally 9+2 then 9+0 (Figs.7 and 8). Above this region however, additional amorphous material was sometimes present (Fig.12) and the microtubular pattern became irregular (Fig.11) or amorphous (Fig.13). At the surface the cilia invariably showed irregular microtubular

structure.

`Stabchen' Receptors On Spines.

Various spines were examined with the scanning electron microscope and a fairly sparse distribution of `stabchen' like receptors found. These receptors sometimes had a cilium projecting 3 or 4µm (Figs.14 and 15)) but in most cases the cilium either did not project from the ring of specialized microvilli or only for a fraction of a micron. The `stabchen' with the larger cilia were most numerous on the large lateral spines and present on only the last 30 distal segments of the arm. In the species under investigation, Ophiura ophiura, only a single cilium was associated with each ring of microvilli to form a stabchen but in A.squamata, Whitfield and Emson (1983) described multiple cilia in some receptors. Transmission microscopy in horizontal section at the surface showed the cilium to be either absent or have an irregular array of microtubules (Fig.16).

In these `stabchen' type receptors satellite material from the main basal body radiated out to the base of the modified microvilli. These microvilli then formed a canal. The `stabchen' projected from the surface with a double array of microvilli; the inner ones of which were derived from the receptor cell and were surrounded by a layer of cuticle which was flanked by

normal, usually detached, microvillous caps. This has been described in detail by Whitfield and Emson 1983. The microvillous canal may, or may not contain a cilium. This cilium was usually amorphous in stucture and never contained a regular 9+2 configuration near the surface.

A Ciliated Structure Beneath The Cuticle Of The Arm.

A previous study (Stubbs & Cobb 1981) failed to describe any possible structures associated with the physiologically documented dermal light sense (see Moore and Cobb 1985a). A further examination was carried out in the present study on the aboral surface of the arm tips. This region is known to be the most sensitive to photic stimulation (Stubbs 1982a). The study revealed a ciliated stucture beneath the cuticle that differed in stucture from those described on the spines and tubefeet. This ciliated stucture which may function as a possible receptor, was characterized by a modified cilium (Figs.17 and 18). This cilium projected from the cell surface for less than 0.5µm and the membrane appeared to be attached to fibrous material found below the cuticle. This fibrous material has been described in detail in this ophiuroid by Holland and Nealson (1978) and is composed of fibres which run parallel to one another in discrete bundles which make up the thick but discontinuous layer of the cuticle. The vast majority of the microvillous caps present were detached from the cells and the cell surface lay between 2-5µms below the

cuticle. The present study has shown that in the smallest segments of the arm the putative receptor cells were only a micron below the cuticle but nearer the disc there were several microns separating them.

These putative receptors lacked a significant ciliary root and were associated with lamellae of membrane in the region of the cilium. The association between these modified cillia and the lamellae was consistent. They were much more difficult to locate in larger more central segments of the arm. There is no physiological evidence for the function of these putative receptors but a possible function based on their structure is discussed on page 85. It is argued that they may function as possible photoreceptors.

Neuronal Connections.

Small bundles of axons (Fig.19) occurred beneath all regions of epithelia examined from tubefeet, spines and the general body surface. They were characterized by a regular array of microfilaments, small mitochondria and the regular packing of the axons into a bundle separated by an unspecialized but fairly uniform cleft. No attempt was however, made to show that any of these axons were derived directly from the receptor cells.

Echinoid Cilia.

The irregular structure of the cilia at the surface which did not show a normal 9+2 configuration could possibly have been a fixation or decalcification artefact. A number of cilia taken from the modified buccal podia of Echinus esculentus, prepared using the same fixation methods, showed unequivocally the presence of a 9+2 structure (Fig.20). It is unlikely that the irregular structures of the cilia encountered in the ophiuroids were therefore artefacts of any kind.

FIG. 1.

Scanning micrograph of part of tubefoot from Ophiura ophiura. Numerous secretory pores are apparent and small raised structures (arrows) resemble stabchen described by Whitfield and Emson (1983).

Scale = 10μm.

FIG. 2.

High magnification of similar area to Fig. 1. Numerous secretory pores are present and occasional raised stabchen (single arrows). Other small structures (double arrows) are normally associated with pairs of secretory pores and transmission electron microscopy has shown these to be modified cilia.

Scale = 1μm.

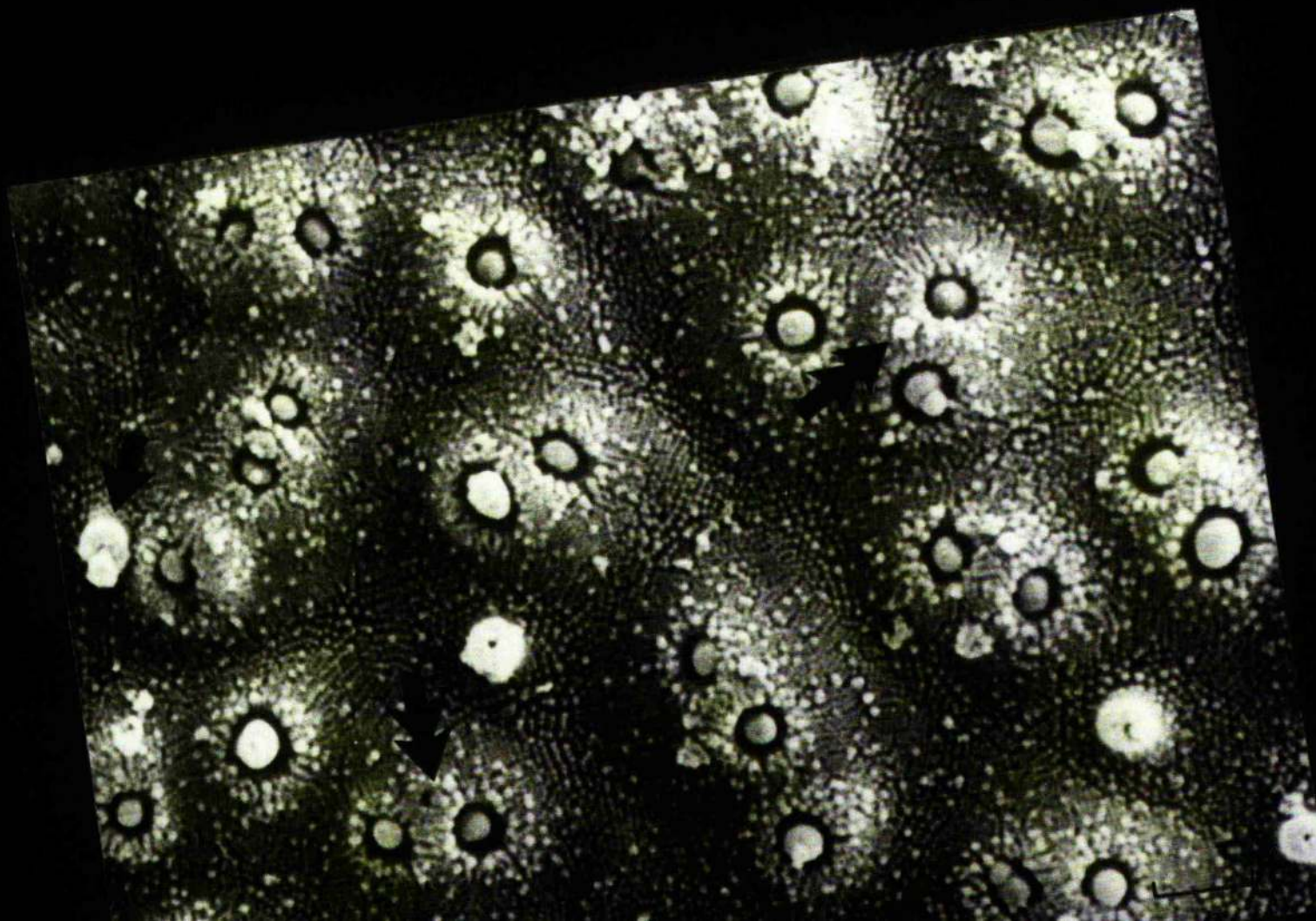


FIG. 3.

Transverse section of a tubefoot showing secretory cells releasing packages of material through surface pores. A raised stabchen type ciliated structure is shown (single arrow). Another ciliary structure (double arrow) is present which does not protrude above the cuticle (c) in this section.

Scale = 1μm.

FIG. 4.

Longitudinal section through stabchen-like ciliary structures. (mt) This presumed receptor structure is similar to that described by Whitfield and Emson (1983) on brittlestar spines. These cells are closely associated with secretory cells (s), and are joined to adjacent cells by septate junctions (arrows). C is the cuticle.

Scale = 1µm

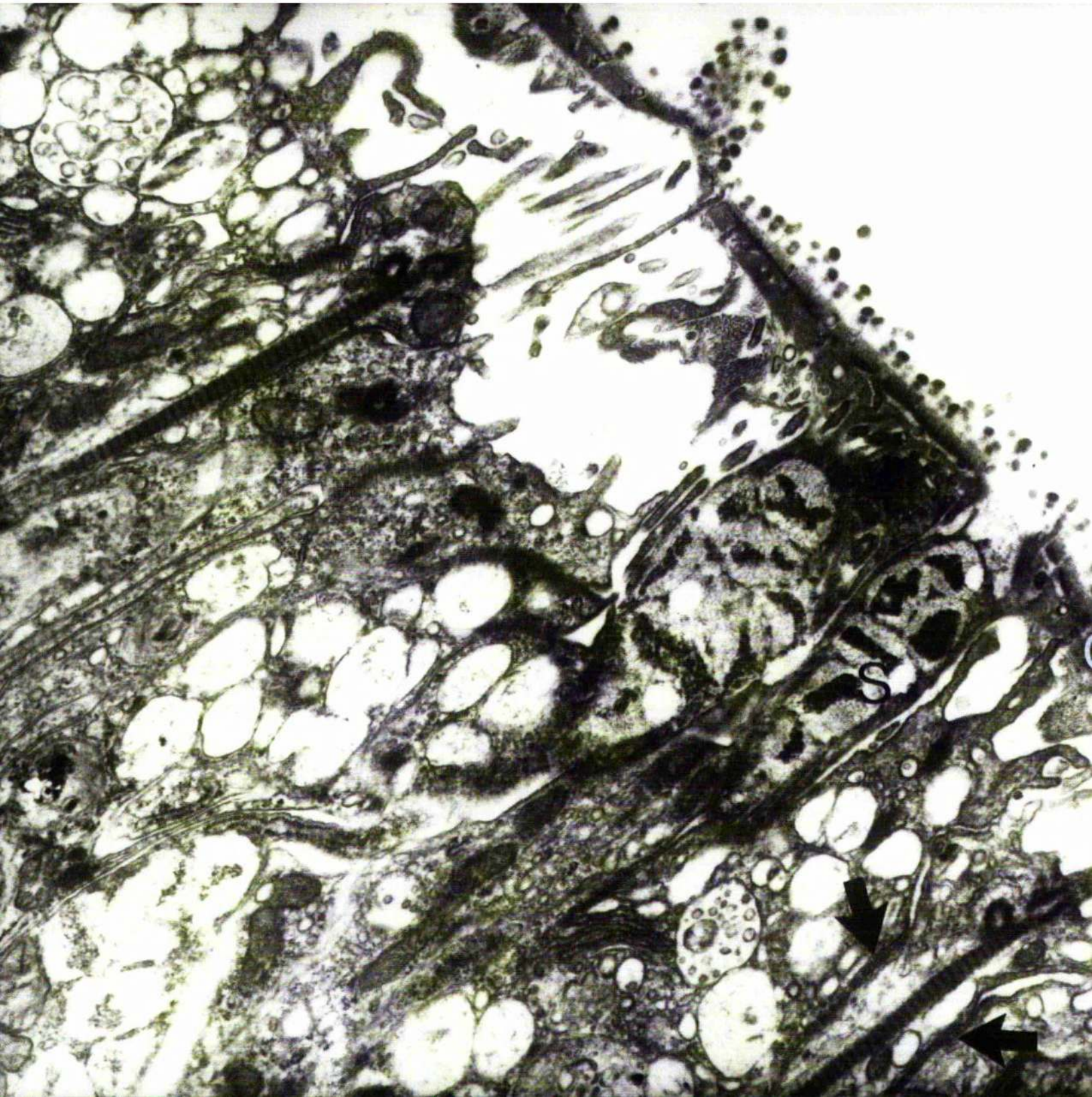

S

FIG. 5.

Longitudinal section through neck of a secretory cell (S). These cells are characterized by a pallisade of microtubules which run longitudinally just beneath the cell membrane (m). Each cell is joined to those adjacent by an intermediate junction (i) at the surface and a deeper band of septate junction (Sj).

Scale = 0.5μm

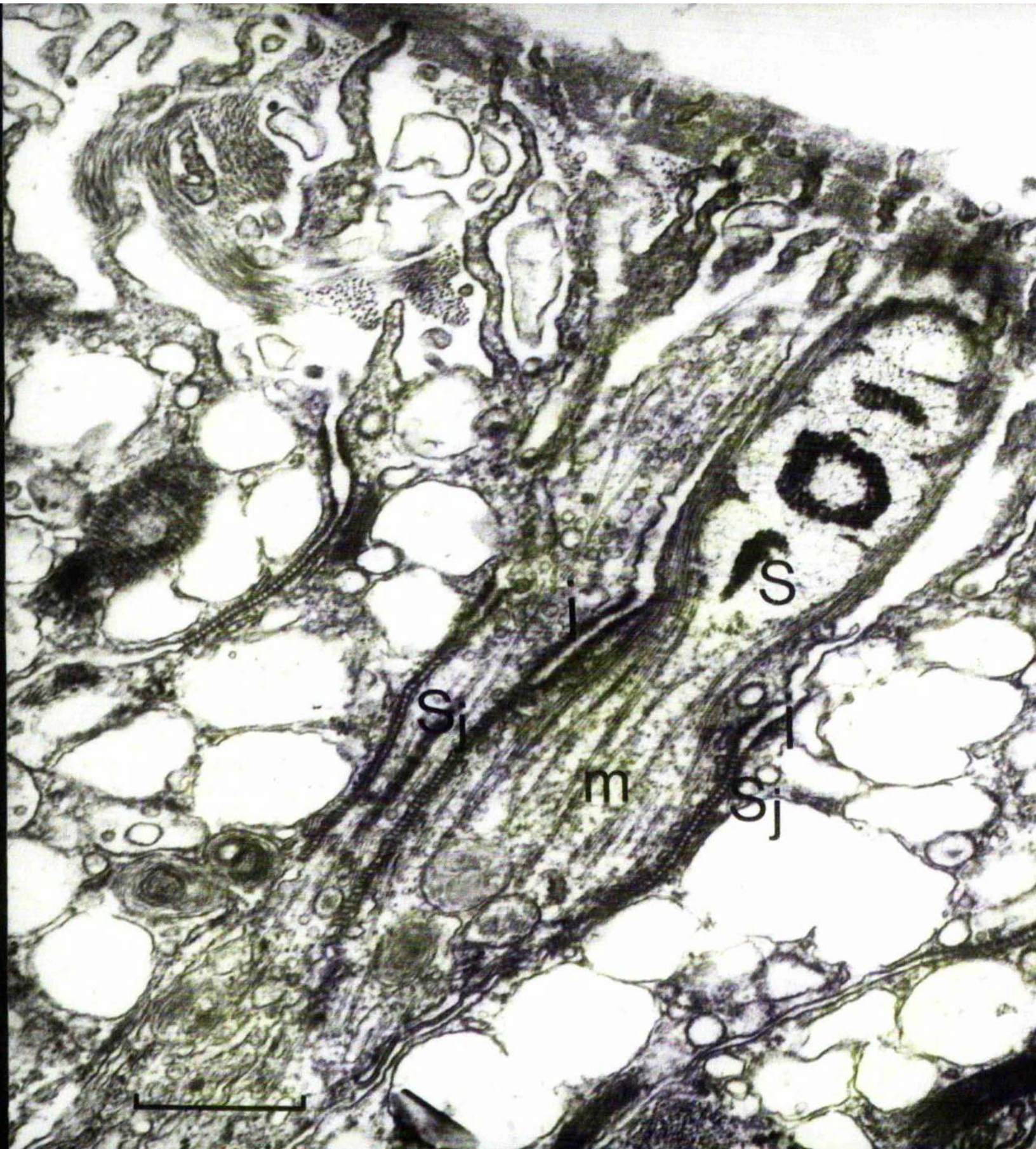

s
i
s_j
i
m
s_j

FIG. 6.

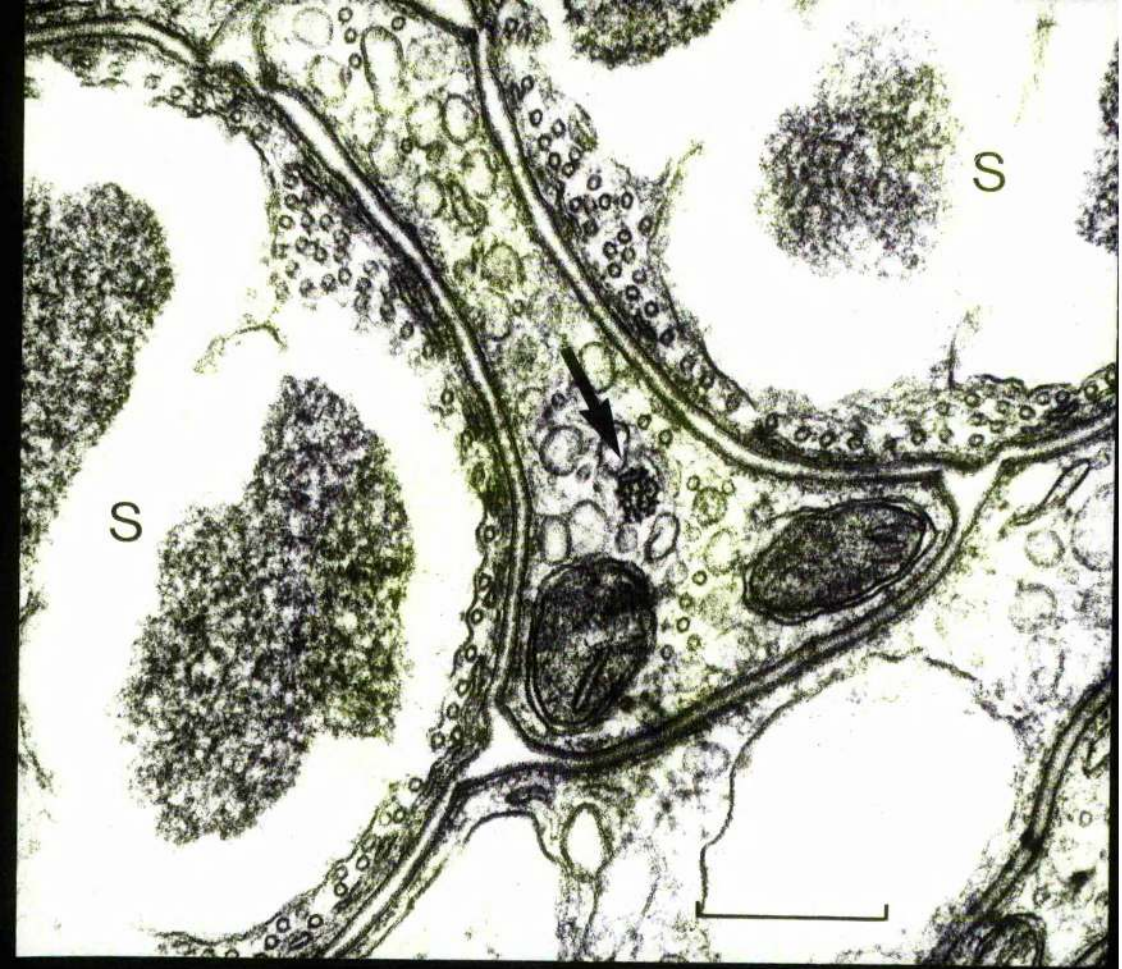
Transverse section through two secretory cells (s) showing characteristic peripheral microtubules. There are presumed receptor cells, usually two, associated with each pair of secretory cells. These receptor cells also contain microtubules and a cilium. This section shows part of one receptor cell at the level of the ciliary root (arrow).

Scale = 0.2μm.

FIG. 7.

Similar section to Fig. 7 cut nearer the surface. A clear 9+2 cilium structure surrounded by microvilli (mv) is seen and another cilium (arrow) is cut in glancing section in a second receptor cell. Two secretory cell(s) and two receptor cells arranged in this fashion is the typical arrangement.

Scale = 0.2μm.


S
S

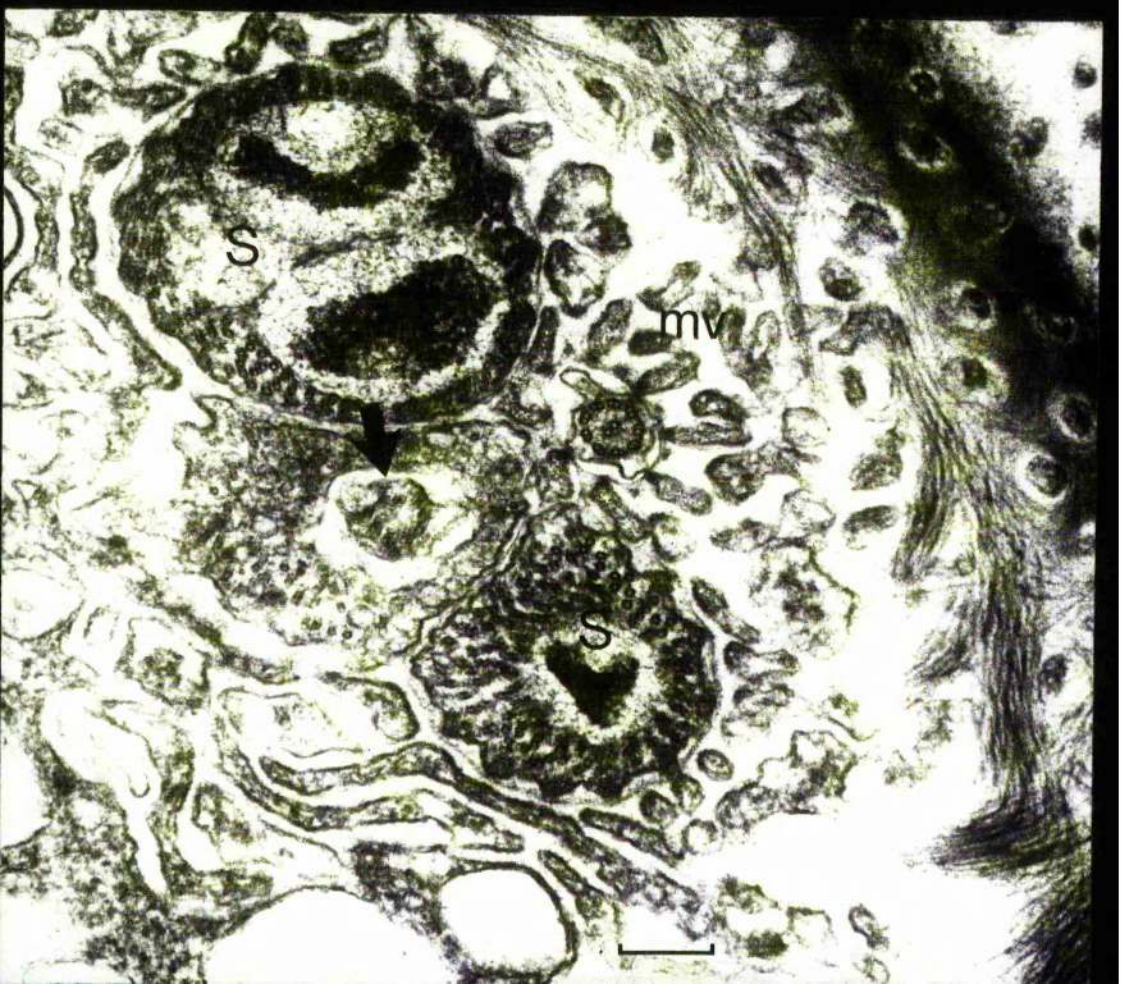

S
mv
S

FIG. 8.

Cilium between two secretory cells (s) showing 9+0 structure from a brittlestar tubefoot. Almost no cilia show a 9+2 structure above the receptor cell surface.

Scale = 0.2μm.

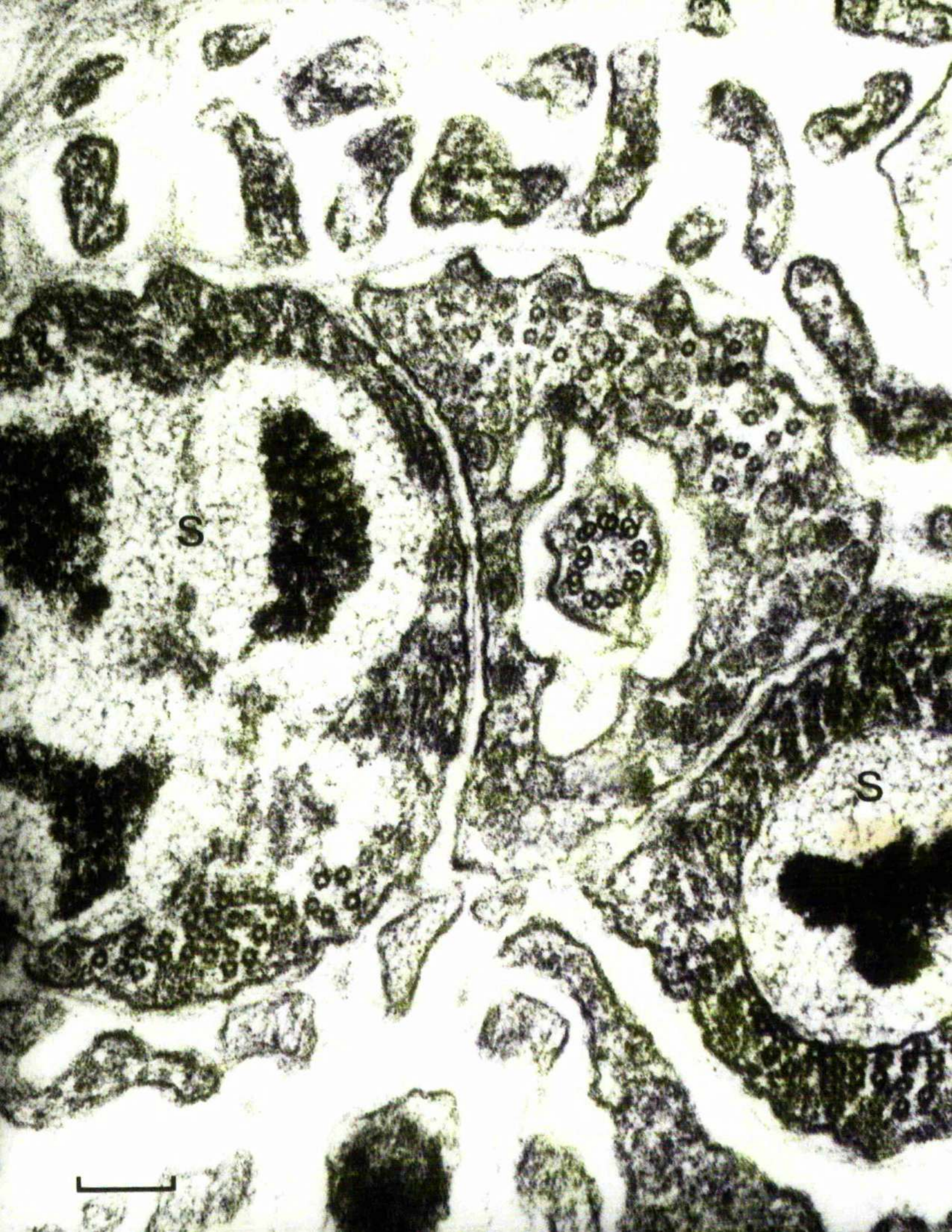

S
S

FIG. 9.

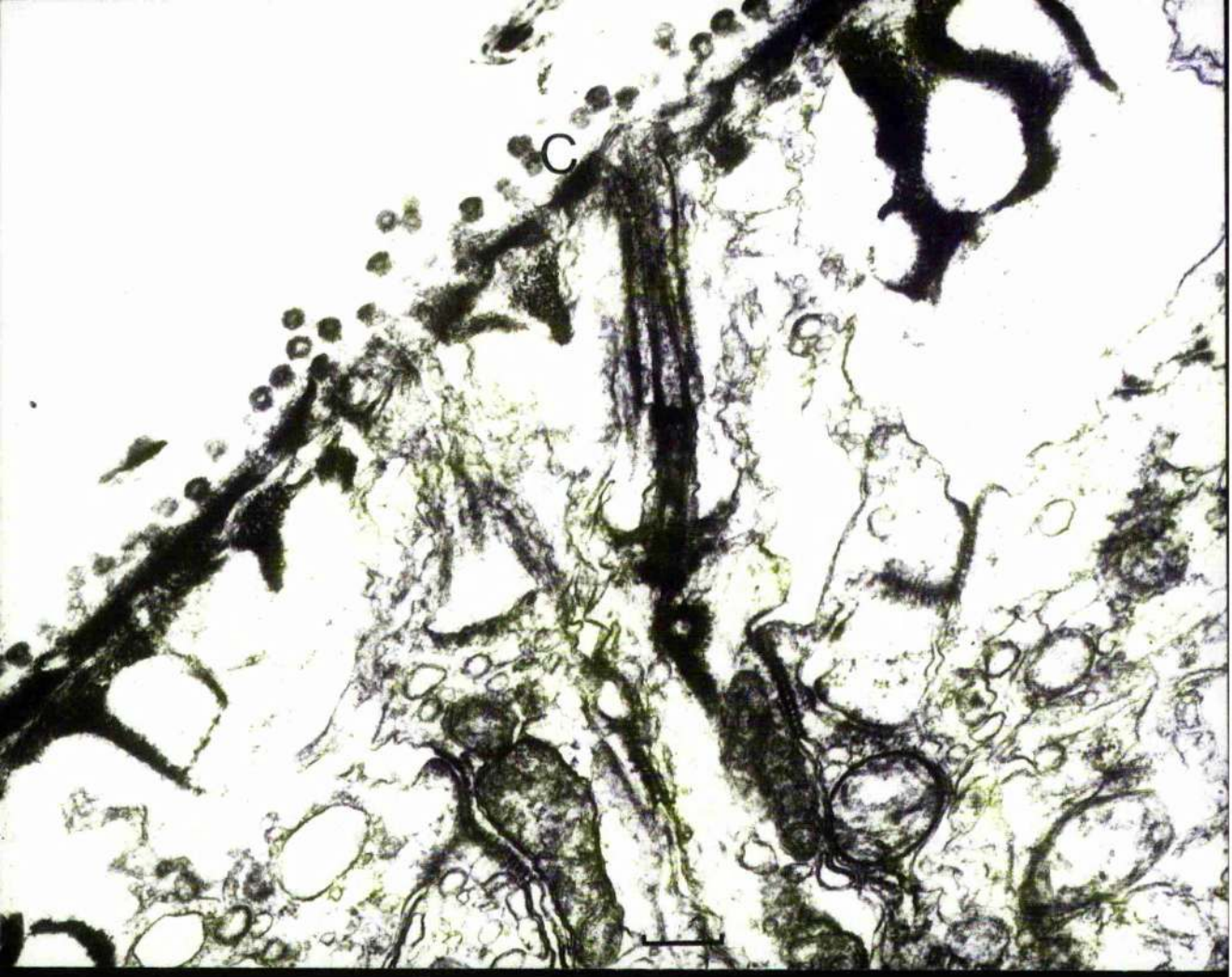
Longitudinal section through non projecting type receptors. Note irregular microtubular array towards cuticular surface (c) where the structure terminates. This structure is not surrounded by modified microvilli when viewed in transverse section.

Scale = 0.1μm.

FIG. 10.

Transverse section through non projecting receptors at tubefoot surface of brittlestar. Two secretory cell pores (s) are shown surrounded by microvilli (mv). Two cilia with indistinct and irregular arrays of microtubules are present between the secretory cells (arrows). These are not surrounded by a channel formed from microvilli as in stabchen type receptors.

Scale = 0.2μm.


C

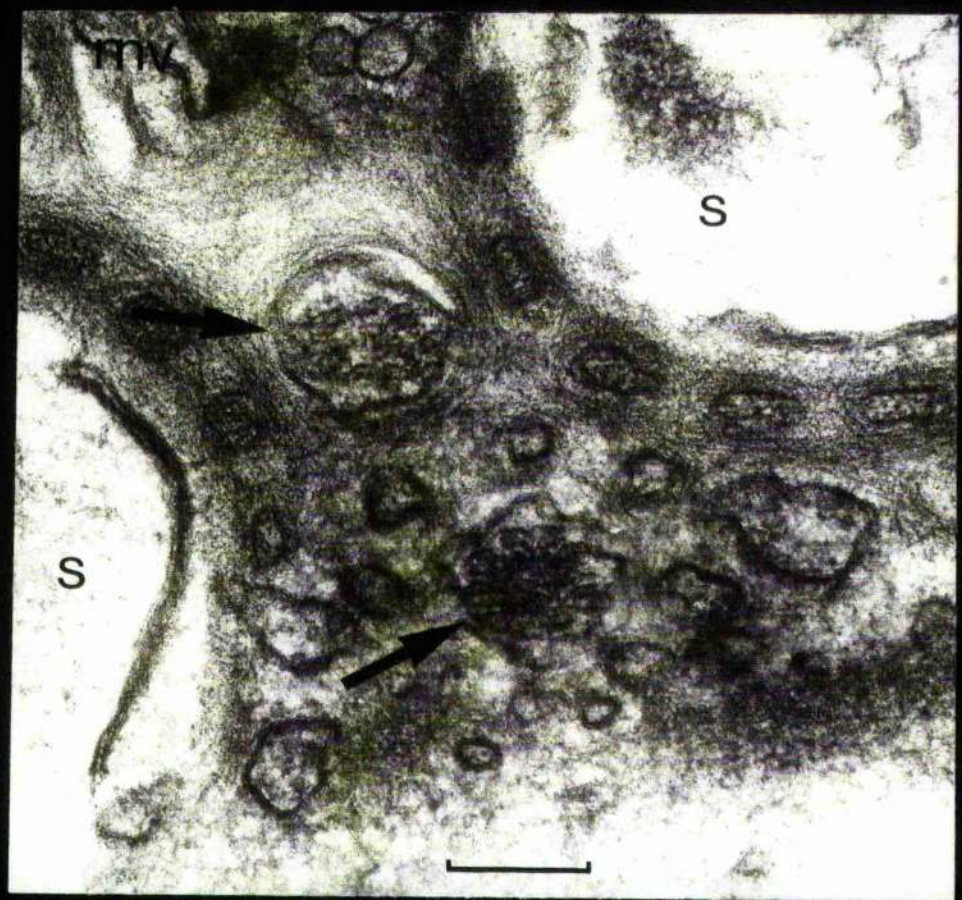

mv
S
S

FIG. 11.

Irregular arrangement of microtubular elements near the surface in non projecting type receptors on the tubefoot of a brittlestar. This is not an artefact of fixation.

Scale = 0.2µm.

FIG. 12.

Transverse section through a cilium of a non projecting type receptor showing additional amorphous material present (arrow) and a 9+0 arrangement of microtubules.

Scale = 0.2µm.

FIGS. 13.

Irregular arrangement of microtubular elements of a cilium near the surface in non projecting type receptors on the tubefoot of a brittlestar.

Scale = 0.2µm.

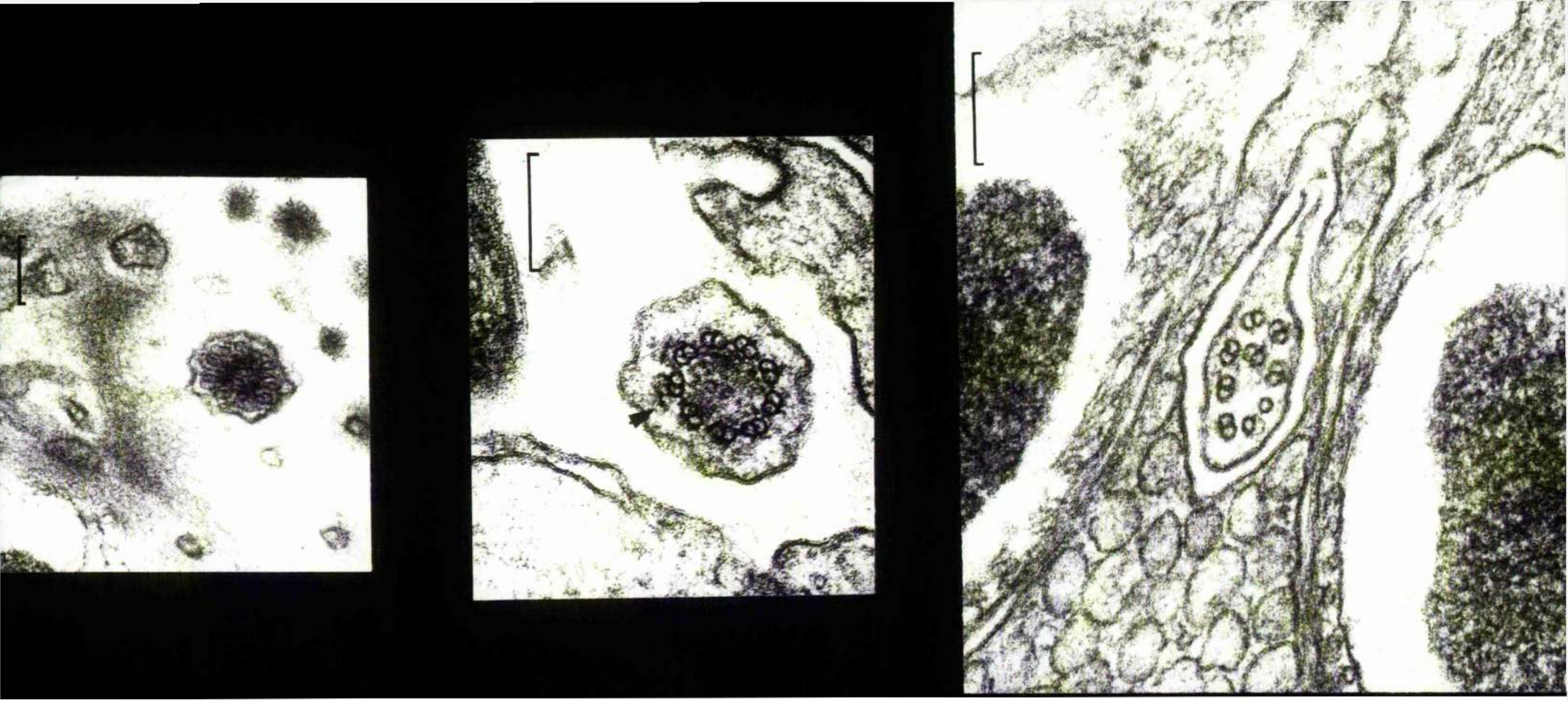


FIG. 14.

Scanning micrograph showing stabchen type receptors with (single arrow) and without (double arrow) protruding cilia on one spine of a brittlestar. Such receptors have a thinly scattered distribution and most do not have a cilium protruding.

Scale = 10μm.

FIG. 15.

Stabchen type receptor with protruding cilium.

Scale = 0.5μm.

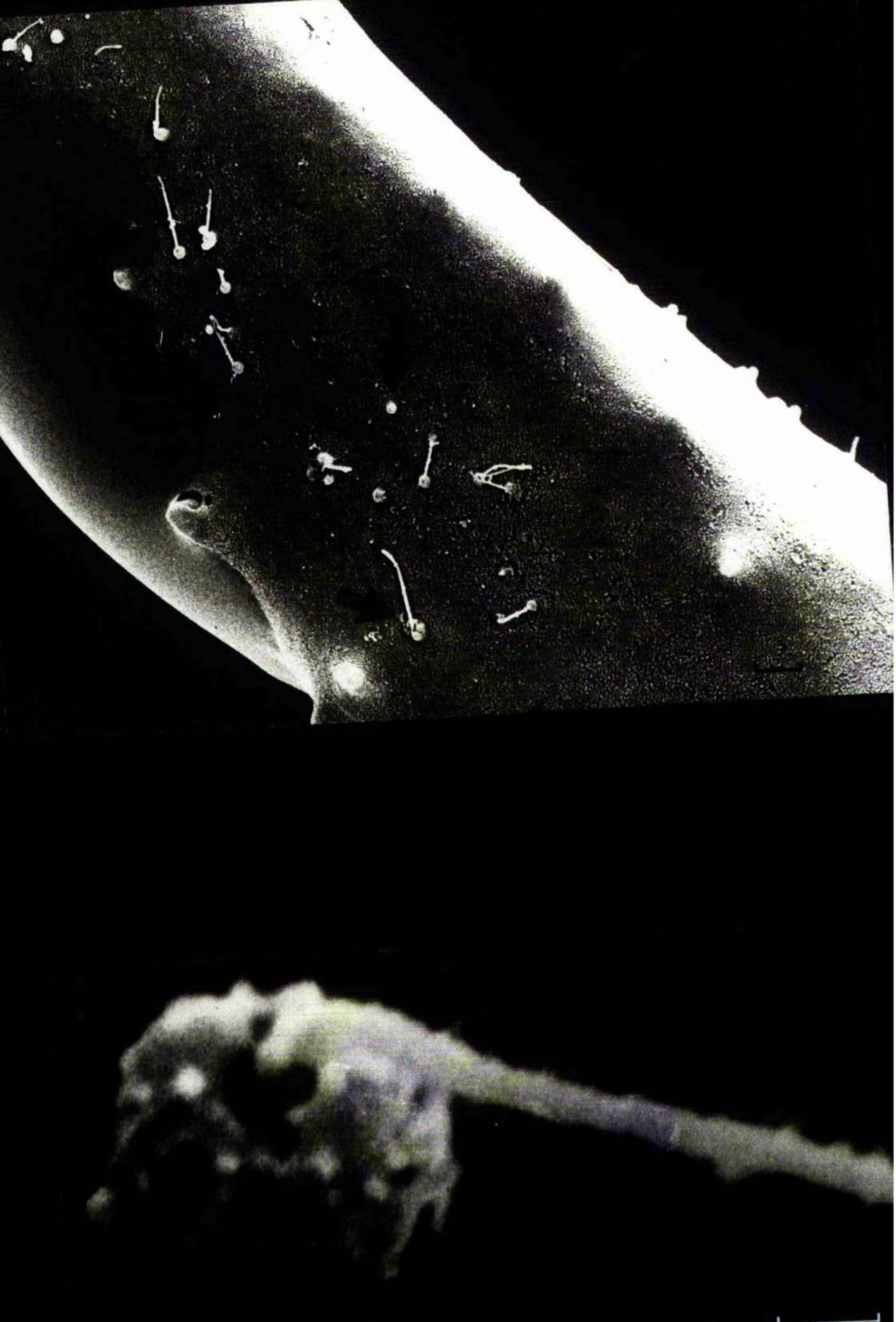


FIG. 16.

Horizontal section through a group of stabchen in decalcified material from a spine. The channel in the centre of the organelle is formed by microvilli (see Whitfield and Emson 1983). The channel formed by the microvilli is clearly seen. One is empty (solid arrow), another contains a 9+0 structure (open arrow), and a third an irregular array of microvilli (double closed arrow).

Scale = 0.5μm.

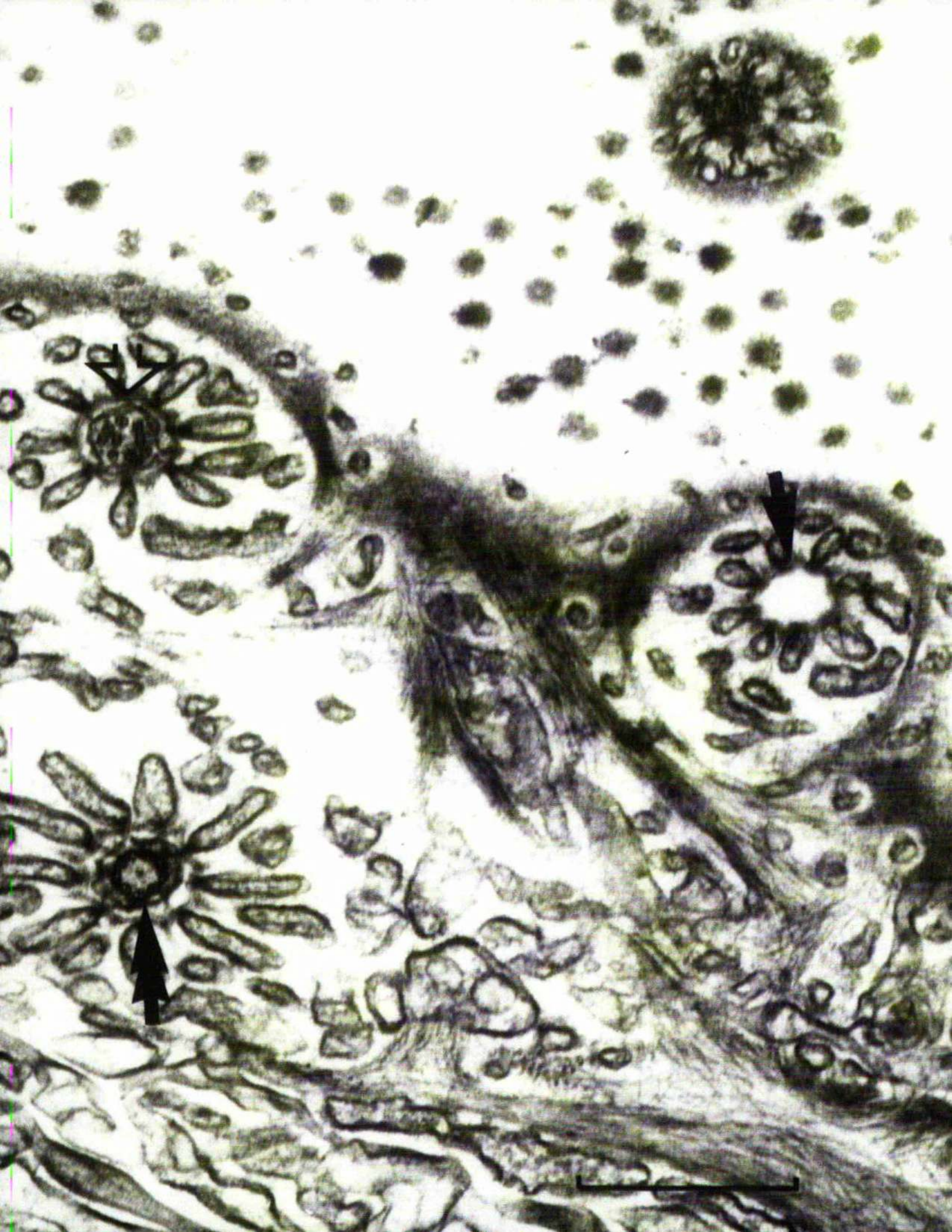


FIG. 17.

Section through decalcified aboral surface of a tip segment of the arm. A modified cilium is seen below the typical ophiuroid cuticle (c). The cilium only just protrudes above the cell surface without microvilli. The cilium membrane appears to be closely associated with bundles of extracellular filamentous material (arrow). This material has been described in this species by Holland and Nealson (1978). The ciliary structures are common and are characterized by a short ciliary root and the apposition within the cell cytoplasm to membrane lamellae (lm).

Scale = 0.5μm.

FIG. 18.

Similar section to Fig. 17 showing the same characteristic features and the associated membrane lamellae (lm). These structures do not protrude above the surface of the cuticle (c) and may in fact be several microns beneath it. Arrow indicates the association of the cilium membrane and the extracellular fibrous material.

Scale = 0.5μm.

Inset: A higher magnification showing the close association between the cilium membrane and the fibrous material.

Scale = 0.1μm.

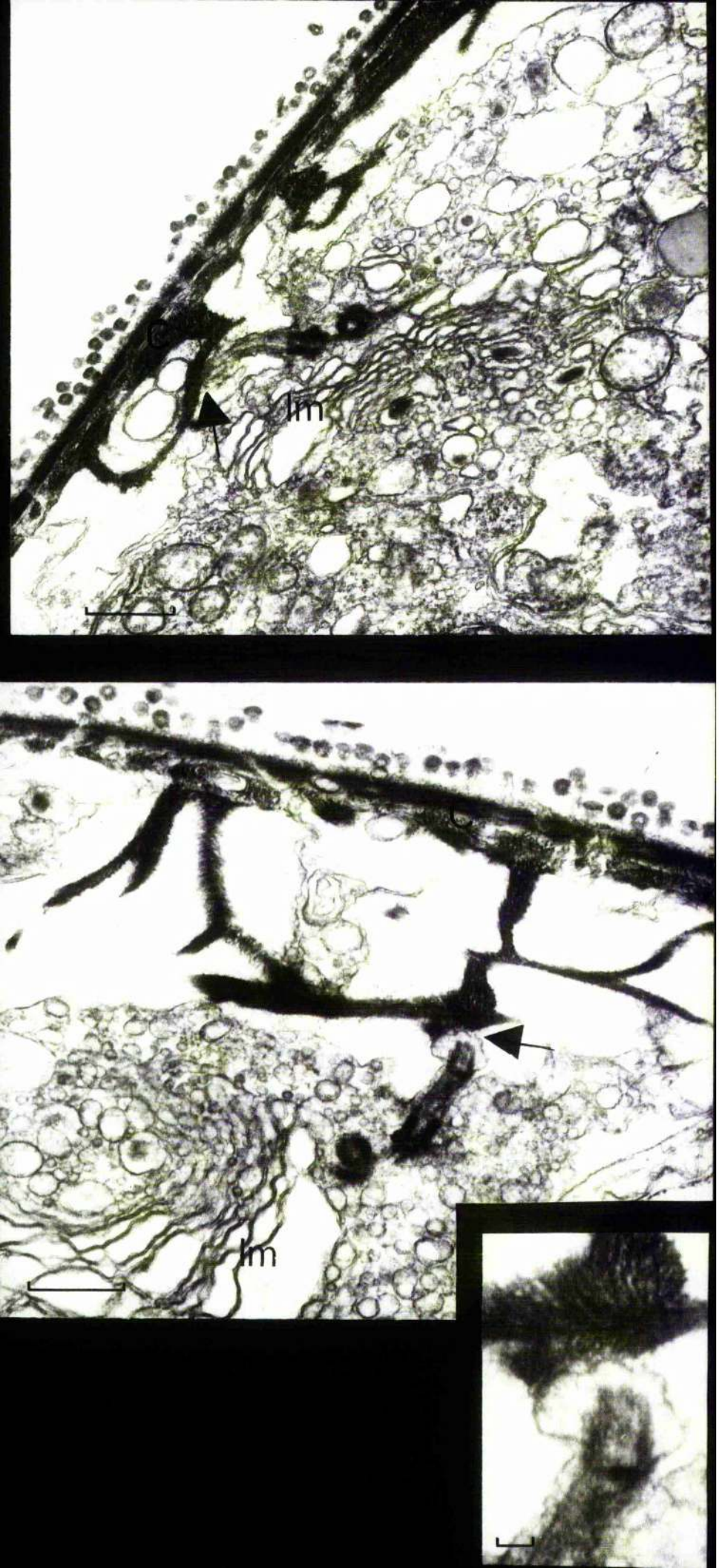

C
lm
C
lm

FIG. 19.

There are numerous bundles of small axons (ax) characterized by small mitochondria and microtubules beneath the epithelium. Processes from some epithelial cells contain filamentous material (fm). These bundles of axons may be sensory, motor or interneuronal.

Scale = 1μm.

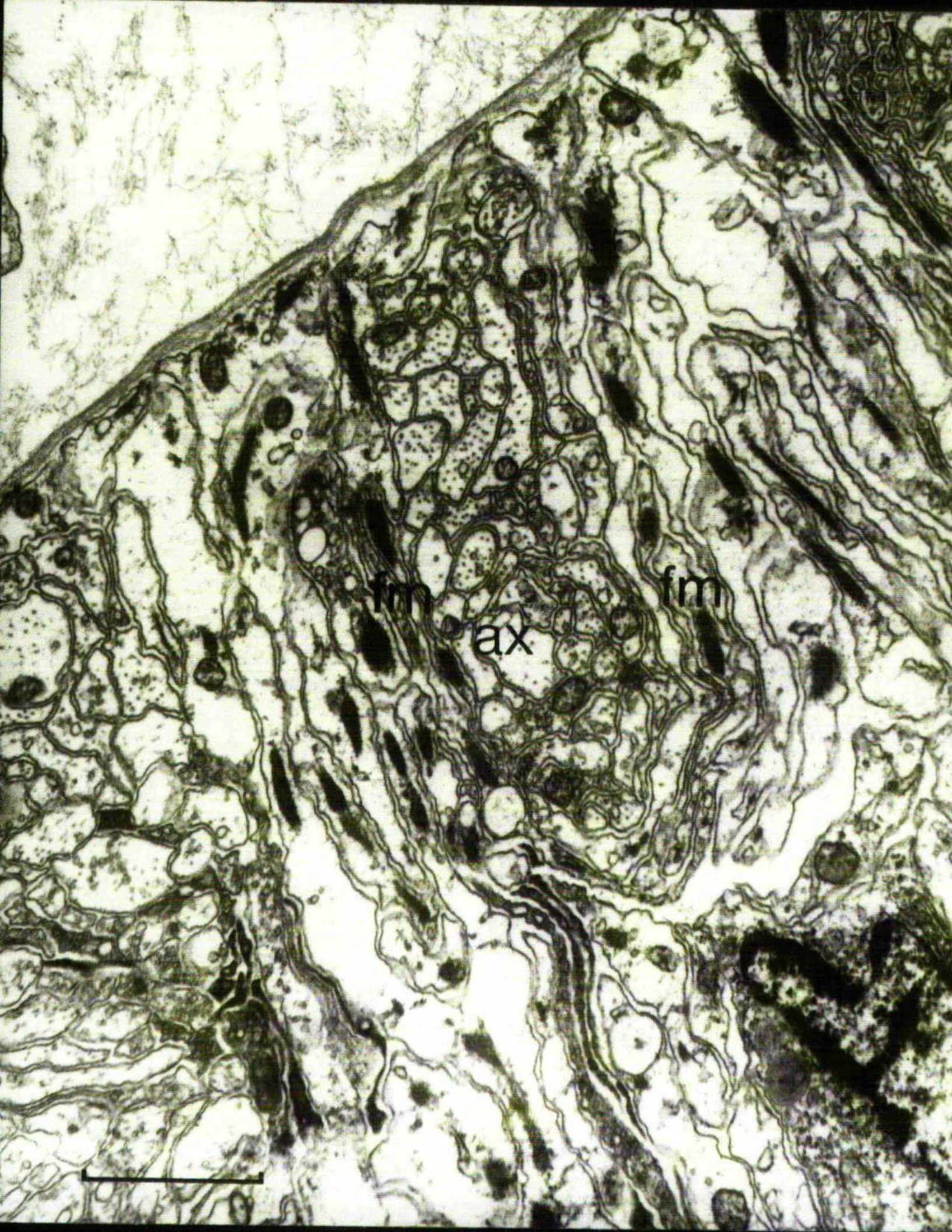
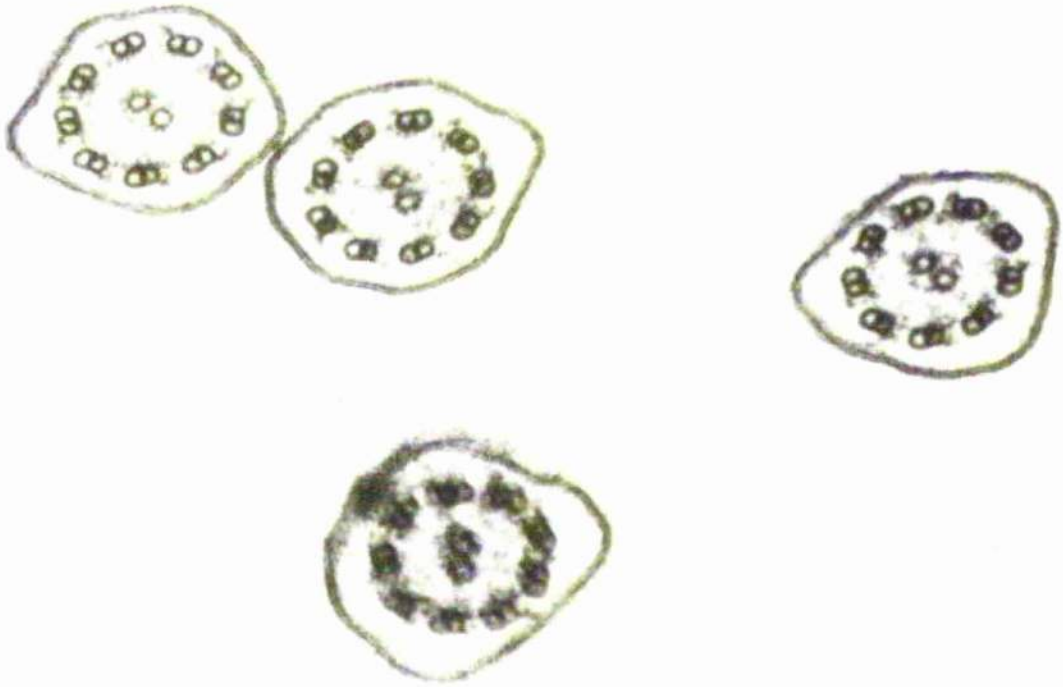

fm
ax
fm

FIG. 20.

Normal 9+2 structure of cilia of Echinus cut in transverse section Tubefeet from Echinus and Ophiura were fixed simultaneously and the irregular structures of the microtubular arrays in the cilia of the latter are unlikely to be fixation artefacts.

Scale = 0.2μm.



DISCUSSION.

In the echinoderms very little is known with any certainty about receptor function. In general sensory nerves are too small to record from and specialized receptor organs are rare. The present neurophysiological study has shown O.ophiura to have receptors located peripherally with considerable sensitivity to photic, chemical and mechanical stimulation. Scanning electron microscopy, and improved fixation techniques for transmission electron microscopy, have recently provided much new evidence on the distribution of presumed sensory receptors over the general body surface of this species.

Many of the presumed receptors reported in this study had a cilium associated with them. These were different from motile cilia which are common within the echinoderms and which contain a 9+2 arrangement of microtubules and measure 10 or more microns in length. Each motile cilium is described as being surrounded by a ring of microvilli and the structure is very distinctive when viewed using the scanning electron microscope (Wood and Carey 1981, Florey and Cahill 1982). Stubbs and Cobb (1982) recently described an epithelial ciliary feeding organ in which the cilia showed a similar structure, but differed in that satellite material from the centriole was associated with the base of the surrounding microvilli. In larval echinoderms it is suggested that

these motile cilia are innervated (Strathmann 1972 and Burke 1978) but there is no evidence for this in adult echinoderms.

Pedicellariae have been shown to respond to both chemical and mechanical stimulation (see Campbell 1973). Cobb (1968) and Oldfield (1975) described the presence of receptor cells with cilia in similar pedicellariae. These cilia had a normal 9+2 arrangement surrounded by modified microvilli, which were linked by satellite material to the centrioles. The cilia were 10µm or more in length and not generally motile although they are capable of movement (Campbell, personal communication). Only one type of cell was present, though the density varied and thus it is possible that these receptors are sensitive to both chemical and mechanical stimuli.

Whitfield and Emson (1983) have comprehensively described a receptor on the spines of Amphipholis squamata and shown these are the 'stabchen' first described by Reichensperger (1908). These receptors possessed a short cilium with a 9+2 arrangement of microtubules. These are similar to those described in the present study and the complex structure described by Whitfield and Emson (1983) which is formed by the microvilli is clearly of importance. The lengths of the cilia in O. ophiura varied and in some cases did not protrude further than the fused tube of microvilli surrounding them. A 9+2 structure was probably rare except immediately above the basal body. In general the

structure is one of irregularly arranged and incomplete microtubules. Removal of the epithelium including the `stabchen' with bleach did not destroy a mechanoreceptive function in spines which may indicate that there is an interoreceptor concerned with mechanosensitiviy present.

The ultrastructure of the tubefeet has been described in all the classes of echinoderms. In general, receptors are present and appear to be closely associated with mucous or other secretory types of cells. Mucous cells have been described in starfish by Souza Santos (1966) and more recently by Martinez (1977) who described six types in the tubefeet of Ophiothrix fragilis. Burke (1980) described receptors in the tubefeet of the larvae and adults from a number of species of sea urchins. These had typical cilia that projected 3-4μm from the surface and were surrounded by a ring of microvilli. Bouland et al (1982) described short cilia on receptors in the holothurian Holothuria forskali and proposed a possible chemoreceptive function. Byrne and Fontaine (1983) showed `stabchen' type receptors in the crinoid Florometra serratissima and noted their close association with secretory cells. Martinez (1977) described cilia with a 9+0 arrangement in the tubefeet of O.fragilis. These resembled the receptors, which do not protrude beyond the surface as described in the present study.

The present study has shown, in both the spine and tubefoot receptors, that the microtubular arrangement above the cell surface was not 9+2, but irregular. This was true of the receptors where the cilia did not project and the `stabchen' type. It was thought possible that this was a fixation artefact, but examination of the cilia from the echinoid buccal tubefeet using the same technique shows that the structure was invariably 9+2. Two types of presumptive receptors, other than the possible receptor described below, are therefore present in O.ophiura. `Stabchen' receptors of fused microvilli often containing a modified cilium which may not project and modified cilia without the specialized collar of microvilli that did not project beyond the cuticle. All are characterized by long ciliary roots. It was not possible to ascribe chemoreceptive or mechanoreceptive functions to any particular structure.

Relatively little is known of the structures of photoreceptors in echinoderms. Eakin (1966) suggested that the membranes of the photic receptors in the ocelli of asteroids were derived from expansions of cilia. Penn and Alexander (1980) proposed that the microvilli in the ocelli of the asteroid Nepanthia belcheri were not derived from the cilia that are present, but from the general cell surface. The function of the ciliated structure, described on page 79 and presumed to be a possible receptor, has not been identified using physiological techniques. There are however, several

features which indicate that this structure could function as a possible photoreceptor. An important piece of circumstantial evidence for such a function is the high degree of photosensitivity of the aboral surface of the arms, particularly the tip region, in this ophiuroid, (Stubbs 1982a, and Moore and Cobb 1985a). Despite the present detailed anatomical study and a previous study by Stubbs (1982a), no specialized structure other than those discussed have been described. The ciliated stuctures do not project through the cuticle and thus could not function as chemo- or contact mechanoreceptors. The structures could function as pressure sensors but they have a structure that is dissimilar to other such receptors (Laverack 1976). There is no behavioural or physiological evidence for the presence of a pressure receptor in this position on the animal. Photoreceptors in other invertebrate phyla have been described by Eakin (1972). He divides these receptors into two types. Firstly ciliary receptors which have lamellae present associated with the cilia membranes. Secondly rhabdomeric photoreceptors which are arrays of microvilli or lamellae. In the present study, there is a substantial membrane system present, close to the cilium, but it is not clear whether this is formed by the folding of the cell membrane or intracellular cytoplasmic membranes. There is a possibility that the lamellae like structures are Golgi membranes. The position of the membrane system close to the basal bodies of the cilia and the peripheral location in the cell, away from the nucleus, is however, not typical of Golgi bodies in this

ophiuroid (see Stubbs and Cobb 1982). A detailed examination using histological techniques, will be required to show whether these membranes contain pigments associated with a photoreceptor function, or enzymes associated with Golgi function. Finally, the cilia consistently lack a long ciliary rootlet. This differs from all known presumptive mechano- and chemoreceptors described in this and other studies. (Burke 1980, Bouland et al. 1982, Whitfield and Emson 1983 and Byrne and Fontaine 1983). The significance of the short ciliary rootlet in terms of transduction mechanisms is, however, not understood. When taken together, it is suggested that these features may indicate that this structure is involved in photoreception. More substantial evidence for such a function would involve an ultrastuctural immunological examination for known photically active pigments.

Cilia with unusual structures have been reported in two other studies. Gardner and Reiger (1980) have shown a modified cilia with a 9+0 arrangement on the endothelial cells lining the tubefeet coelomic cavity. They proposed that these may have a direct stretch receptor function independent of the nervous system. It is interesting to note that Strathmann et al (1972) considered the possibility of epithelial conduction to account for the reversal in ciliary beating in larval echinoderms. Cobb and Stubbs (1981, 1982) described cilia that were present on the cell bodies of some ectoneural and hyponeural neurones. It is conceivable that these

might have been associated with some receptor function and from their internal position this was likely to be proprioreception. These cilia appeared to be non motile.

It is not clear whether all receptor cells are ciliated and there are two lines of evidence that are pertinent. Kawaguti and Kanatani (1964) originally proposed that all epithelial cells in echinoderms were sensory and Cobb (1968) later proposed that this was the case on the pedicellariae of E.esculentus. Markel and Roser (1983) proposed that there was only one epithelial cell type on the spines of Eucidaris tribuloides. This implies either there was no receptor function to the spines or alternatively it too was covered only by receptor cells and Weber and Grosmann (1977) in their study showed a mixture of ciliated sensory cells and non ciliated epithelial cells, the latter being characterized by the presence of tonofilaments. The second line of evidence was provided by Bouland et al (1982) and Fankboner (1978) who suggested that the papillary cells of tubefeet and tentacles in holothurians were covered in microvilli but lack ciliary structures may have been receptors of the gustatory type. Microvilli are clearly modified and involved in the structure of many of the supposed ciliated receptor cells. It is possible, that in some cases only microvilli are present in receptor cells.

There is some evidence that neurones function as non-specialized receptors. Millott (1968) suggested that certain neurones in echinoids may be photosensitive and Millott and Colman (1969) described a structure named the podial pit where a substantial but unspecialized plexus of nerve fibres lay beneath the epithelium. These authors suggested that these might be photoreceptive. Physiological studies on O.ophiura are the only unequivocal evidence of photoreception and Stubbs (1982a) indicated that the nerves in the radial cord were not photosensitive. However, this does not exclude the possibility of the peripheral nerves being so. This study has shown that individual spines were sensitive to water borne vibration and mechanical displacement, even with the epithelium removed with bleach. This implies that there were receptors within the ligaments which monitor stretch. Lewis (1968) reached the same conclusion after a study of the ultrastructure of the echinoid sphaeridia which function as supposed gravity detectors. An examination of such ligaments (Cobb unpublished) showed a number of very different types of nerve fibres but no specialized endings. It may be concluded that it is possible that there exist morphologically unspecialized neurones which have a receptor function.

The relationship of the various types of presumed receptor cells to the nervous system is not clear. In the pedicellariae the receptor cells were unequivocally neurones and sensory axons could be demonstrated as being derived from them. Weber and Grosmann (1977), Burke (1983) and Whitfield and Emson (1983) used serial sections to demonstrate that sensory axons were derived from the receptor cells. Other studies have not indicated this one way or the other, but some suggest a synapse may occur between the receptor cell and the sensory axon. However, this has never been satisfactorily demonstrated. It is difficult to cut serial sections to demonstrate the origin of sensory axons and it has to be accepted that this feature would be easy to miss and difficult to identify in a random section study. In view of the available evidence it may be advantageous to assume that all sensory receptors in echinoderms are neurones that directly give rise to sensory axons unless proven otherwise.

The relationship between presumed receptor cells and secretory cells in some tissueshas been commented upon by a number of workers particularly Whitfield and Emson (1983), Byrne and Fontaine (1983) and De Vos (1985). These cells were separated by septate junctions and Green et al (1979) ascribed a function to these junctions similar to tight junctions in vertebrates. Wood and Carey (1981) have discussed in detail the lack of communicating junctions (gap junctions) in echinoderms

with reference to the tubefeet and suggested there may be another mechanism of communication. It is possible that septate junctions may have some coupling function between cells as well as occluding extra cellular space.

The presumed receptor cells reported in this study were widespread over the spines, podia and aboral areas of the arms in O. ophiura. In many of them the ciliary structure was modified from 9+2. It was not possible to ascribe a particular function to these receptors based on morphology alone.

GENERAL DISCUSSION

DISCUSSION

Any analysis of the sensory discriminatory abilities of the echinoderms, have in the past been based solely on changes in behaviour of either the whole or discrete parts of the animal. The one sensory modality that has received most attention within the literature, namely chemoreception, has been recently reviewed by Sloan and Campbell (1982), who drew attention to the inconsistant and in some cases almost anecdotal results obtained using this approach. The reliance on behavioural criteria has been the direct result of the difficulty of recording electrophysiologically from the nervous systems of the echinoderms. This is due in large part to the small size of the neurones in most classes of the phylum. Brehm (1975) however demonstrated that single unit potentials were recordable from the radial nerve cord of a Californian ophiuroid Ophiopsila californica and subsequently Stubbs (1982a) carried out a preliminary study on Ophiura ophiura, recording activity from the R.N.C. to photic stimuli using suction electrodes. The ophiuroids are suitable preparations for neurophysiological studies due to the presence,(within the nervous system), of `giant' nerve fibres, the layout of which is described in Ophiura ophiura by (Cobb and Stubbs 1981, 1982, Stubbs and Cobb 1981). The presence of `giant' nerve fibres and their significance to the ophiuroid has been previously discussed by Cobb and

Stubbs (1981). The present use of similar neurophysiological techniques to record the responses of O.ophiura to certain environmental stimuli, has led to consistent results being produced which define the sensory discriminatory capacity of the brittlestar. Consistent recordable responses to various levels of stimulation were made possible by controlling the other sensory stimuli present during experimentation and the long term adaptation of each preparation to the experimental situation.

The present study has shown O.ophiura to have a high degree of chemo-, photo- and mechanosensitivity, all three of which provide important information to the brittlestar concerning its environment. The sensitivity to different concentrations of amino acid solutions was greater than that indicated by previous studies on echinoderms. (Reimer and Reimer 1975, Valentincic 1975, 1979, Zafiriou 1972 and Castilla 1972). The levels of detection approached the thresholds demonstrated in fish (Carpio 1978) and littoral crustaceans (Fuzessery and Childress 1975). The sensitivity to sudden decreases in the ambient light level or shadow was very pronounced in O.ophiura and is likely to be of great adaptive significance to the animal. Stubbs (1982a) showed that the animal was also sensitive to light of the intensities and wavelengths found at depths at which brittlestars commonly occur. The sensitivity in the mechanical mode covered a wide range of frequencies not only in the near field region of a vibrating source but through the far

field propagated pressure wave, to the detection of various water currents.

There are two aspects to be considered when dealing with the detection of movement by the brittlestar. Firstly the ability to detect movement of the environment, hydrodynamic disturbances resulting from biotic or abiotic factors. Secondly the detection of movement in the animal itself, its ability to perceive its position in relation to the substratum, ie. proprioception.

It is possible that detection in the near field region of a vibrating source, particularly very low frequencies, may be mediated through deflection of the long projecting cilia located on the distal segments of each arm and described in the structure chapter. The position of the cilia and the low energy input needed to deflect such structures to produce a response suggest detection mediated in this way although no direct evidence of function is available. Other more general movement of the environment is detected by the physical deflection of individual arm spines as a result of hydrodynamic forces acting upon them. Removal of the epithelium and the subsequent inability to diminish the recorded response to water movement, inferred the presence of an interoreceptor in the arm spines which may explain proprio- and gravity perception in echinoderms. Lewis (1968) in his study on sphaeridea in echinoids suggests that in light of the absence of statocyst like

structures, gravity detection is mediated by the pull on ligaments as the sphaeridea is moved. This fits in well with the evidence in the present study, where movement of the animal and the environment, is considered to be detected by deflection of the spines. In O.ophiura deflection of the spines resulting from inversion and detected by an interoreceptor would provide the animal with adequate information concerning its relative position. Reese (1966) has reviewed the substantial literature on the righting response but how the animals sense their inverted positions and the stimuli which release the righting behaviour has always been controversial. The interoreceptor neurone located in the spine may well be unmodified and together with the presence of motor sensory and juxtaligamental nerves makes it difficult to prescribe any function to neurones located, and the small size would provide difficulties in producing physiological evidence.

The distribution of the receptors over the general body surface of all tube-feet and spines in O.ophiura, the presence of a dermal light sense and the failure to localize receptors into discrete specialized complexes, fits in well with the basic segmental plan of an echinoderm nervous system proposed by Cobb (1970). The layout of a nervous and receptor system in such a way allows the brittlestar to undergo autotomy without any great detrement to the survival of the animal. The localization of photo- chemo- and mechanoreceptors into complex organs located on areas of the test that are

readily lost as a result of autotomy would be of adaptive disadvantage. The widespread distribution of receptors and the basic segmental plan of the nervous system permits loss of areas without seriously affecting the functioning of the nervous/receptor systems.

The neurophysiological studies were carried out by recording from the interganglionic region of the ectoneural portion of the radial nerve cord. All extracellular recordings were from inverted preparations to facilitate access to the nervous system which lies close to the oral surface of the animal. Stubbs (1982a), demonstrated that recordings from animals restrained in the more `natural' aboral side uppermost position were similar to those from the inverted position. However, it is not known at present whether recording from the brittlestars in the inverted position affects in some subtle way both the sensory abilities or the integration of the nervous activity within the nerve cords. A comparison of recordings from animals in the aboral side uppermost position in response to various environmental stimuli would be of interest.

All recordings were made from a class of interneurone (Stubbs 1982a), and not from the receptor neurones themselves. Unequivocal evidence from a study on the sensory hillock in pedicellariae (Cobb 1968) has shown in this situation that the sensory neurones are derived directly from the receptor cells themselves. It is suggested that this is the case with sensory neurones

in all echinoderms and discussed in the previous chapter. It is not fully understood whether the receptor cells are located into small localized areas scattered over the body surface or form a significant portion of the whole epithelium. Direct electrophysiological recordings from sensory neurones are difficult because of their small size and difficulty in identifying. Anatomical studies of the neurones in the side branches running from the radial nerve cord to innervate the spines and podia illustrate this (Cobb and Stubbs 1981). Axons are mostly less than 1μm diameter and it is not evident whether the neurones in the side branches are themselves the sensory neurones or an interneurone connecting to the larger longitudinally running neurones in the R.N.C.

It is not apparent either how excitation of the sensory neurones resultsin the firing of the larger interneurones. The inability to record a shadow response when illuminating only one segment (Stubbs 1982a) and the need to illuminate more than five arm segments to produce recordable interneuronal activity to a light 'ON' stimulus suggests that these interneurones may be depolarized only after the required input from a set number of receptor cells is exceeded. Individual sensory neurones may have a lower threshold of detection than is apparent when recording from the larger interneurones. However single sensory neurones may not be sufficient to produce conducted activity within the large interneurones from which the recordings are made and this may only

occur after the summation of the inputs from several sensory neurones. The absolute thresholds of detection are thus impossible to record using this technique. At present nothing is understood of the primary transduction mechanisms in echinoderm receptor systems, how the large longitudinal interneurones are excited and the mechanism by which the interneurone spike train co-ordinates complex behaviour.

The differences between the thresholds of response to amino acids obtained using behavioural criteria and physiological recordings may have been due to the interaction of several factors. Firstly the area available for sensory reception was increased in the behavioural studies when whole animals were used. This may have been sufficient to produce the activity within the large diameter neurones resulting in co-ordinated behaviour. Secondly as discussed above the sensory neurones may well have a lower threshold of detection, which is not recorded physiologically. Lastly observations have shown that the detection of stimulus may not necessarily result in an observable behavioural change.

In addition to the problem of cancellation and summation of units discussed earlier in the work, other factors may affect the interpretation of extracellularly recorded activity in terms of amplitude and number of units present in the response. Consideration must be given to the position and attachment of the electrode on

the radial nerve cord. Although each electrode was relatively large in relation to the width of the nerve cord and the layout of the giant fibres was consistent between preparations, there was always a slight variability in the electrode position. The size of a particular extracellularly recorded spike is dependant upon the size of the neurone involved, its distance from the recording electrode and the amount of current leakage between the neurone and electrode. As there were always slight differences between the degree of attachment of the electrode to the nerve cord, spikes of the same amplitude from different preparations may have been the result of recording from two sized neurones at varying distances from the electrode. Similarly variation in the amount of current leakage and therefore size of the spike recorded from a given neurone would result from differences in electrode attachment.

However, given these considerations it was possible to relate the largest spikes recorded extracellularly with the larger more intense stimuli presented within each sensory modality. Under certain circumstances, the number of smaller potentials decreased and only the larger units were evident within the response. The significance of the reduction in the smaller amplitude spikes within these responses is not understood. The larger spikes were conducted more rapidly than smaller units and dye-filled neurone studies (Cobb personal communication) have recently shown that the neurones from which the potentials were recorded extend as much as

three segments within the radial nerve cords and perhaps further. Intracellular studies have also indicated that within the shadow response, the burst of large spikes is composed of a small number of units within the pattern which fired repetitively (Cobb personal communication). The largest spikes were always associated with suppression of movement in the presence of a possible threat to the animal. The inclusion of a small number of rapidly conducting, long neurones within the basic segmental layout of the nervous system resulting in a more rapid response may be of great adaptive value to the brittlestars. It must be remembered that because of extracellular recording conditions the general 'noise' was of 10μv amplitude and the largest recorded spike only 50μv. As the majority of neurones are small, a significant proportion of smaller spikes that may have been of importance were obscured in the general 'noise' of the response.

The detailed cellular mechanism of integration in the nervous system is not understood. Two basic types of conducted activity may occur. Firstly the response may be integrated in the radial nerve cord ganglion nearest the point of stimulation, resulting in the transmission of an integrated response to all other parts of the nervous system. This integrated response would carry the information resulting in the release of the relevant behaviour in each part of the animal. Secondly stimulation may result in a response that as it passes through the nervous system is continually modified in

different parts of the animal which results in co-ordinated behaviour. This may possibly occur at each radial nerve cord ganglion. Stubbs (1982a) suggests that the former is the case and based his observation on the shadow response. A light `OFF' stimulus produced on the arm tip results in a burst of single unit activity the number, size and pattern of which is similar when recorded from various parts of the R.N.C. in a whole animal preparation. Similar results were obtained in the present study in response to shadow, intense mechanical and single amino acid stimuli. The observation that the response is carried to all parts of the animal in a substantially unaltered form, is based on a response that elicits the same type of behaviour in all parts of the animal, i.e. freezing of movement and which does not necessarily need to be modified as it passes around the animal. However, responses recorded to food items which elicit complex behavioural changes in the form of locomotion are found to be substantially different in pattern when recorded from different positions of the animal. This is evidence that integration of the response which releases different behaviour in different parts of a moving animal is integrated as it passes around the body. Stubbs (1982a) did in fact show minor changes in the pattern of the photic response as it was conducted around the nervous system but prescribed it to differences in electrode position. It is not clear whether separate pathways within the ectoneural nervous system transmitted photically, chemically and mechanically elicited responses or whether the system has

a more general role in which information from a wide variety of stimuli was transmitted via the same pathway.

Cobb and Stubbs (1982) described the anatomy of the ectoneural nervous system which is composed of densely packed neurones of varying diameter running longitudinally along the R.N.C. as much as three segments. These neurones transmit sensory information to all parts of the animals. Tuft and Gilley (1985) propose that these neurones are localized into discrete bundles and that two types are present which they designated as fast and slow conducting neurones. However, Cobb and Stubbs (1981) found no anatomical evidence for this and recent intracellular studies on the ectoneural nervous system (Cobb 1985) suggests that the wide variety of stimuli are transmitted via the same pathway.

The different behavioural responses that were released at various levels of stimulation suggests that there was a hierarchy of behavioural response in O.ophiura dependant upon the level of the relevant sensory input. The observation that the shadow response was never sufficient to produce cessation of activity in nutritionally deprived animals and override the feeding response, suggests a second hierarchy of response that was dependant upon the nutritional and physiological state of the animals. The behaviour of the brittlestars appears to be variable in nature and dependant not only on the interactions between the various environmental modalities providing the sensory information, but also on

the internal state of the individual animals. Two hierarchies of behaviour are therefore evident, the first released various stereotyped responses dependant upon the degree of stimulation by a single sensory parameter. This was evident when increasing concentrations of amino acid solutions and food items were introduced during whole animal experiments. However, this hierarchy itself was dependant upon the internal state of the animal. Nutritionally deprived animals would immediately perform searching behaviour without the usual arm waving or coiling characteristic before appetative behaviour. This variability in echinoderm behaviour has been suggested by Diebschlag (1938) who considered that behaviour was determined not only by physical environmental factors but was also influenced by the physiological or nervous state of the animals. The failure of various workers to take into consideration the complexities of echinoderm behaviour involved when studying the sensory discriminatory abilities of this phylum, using behavioural criteria, may explain the inconsistencies in the literature. Sloan (1982) suggests that interpretation of chemoreception in the echinoderms requires consideration of more variables within the experimental set up, such as an appreciation of their behavioural variability rather than just attraction to the food.

Further work on the interactions between various sensory stimuli on brittlestars maintained at different physiological and nutritional states would be of interest.

The use of extracellular techniques to record from the radial nerve cord has provided an unique opportunity to define the sensory discriminatory abilities of O.ophiura. Definition of how the various environmental stimuli are perceived by the brittlestar and how co-ordinated whole animals behaviour is produced in response to them, would further increase our understanding of echinoderm behaviour. Analysis of both the effects of the levels of excitation already present in the nervous system at the onset of stimulation and the effects of simultaneous stimulation with different environmental parameters may further define the various hierarchies of response. Intracellular studies on the ectoneural and hyponeural nervous systems of O.ophiura are now routine. The study of individual ectoneural giant fibres will permit an understanding of how sensory information is transmitted to all parts of the animals, and their relationship with the motor responses which results in behaviour in the animal.

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PUBLICATIONS.

Much of the work reported in this thesis has been published in the following articles.

Moore, A. & Cobb, J.L.S. (1985) Neurophysiological studies on photic responses in Ophiura ophiura. Comp. Biochem. Physiol., 80A, pp.11-16.

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